





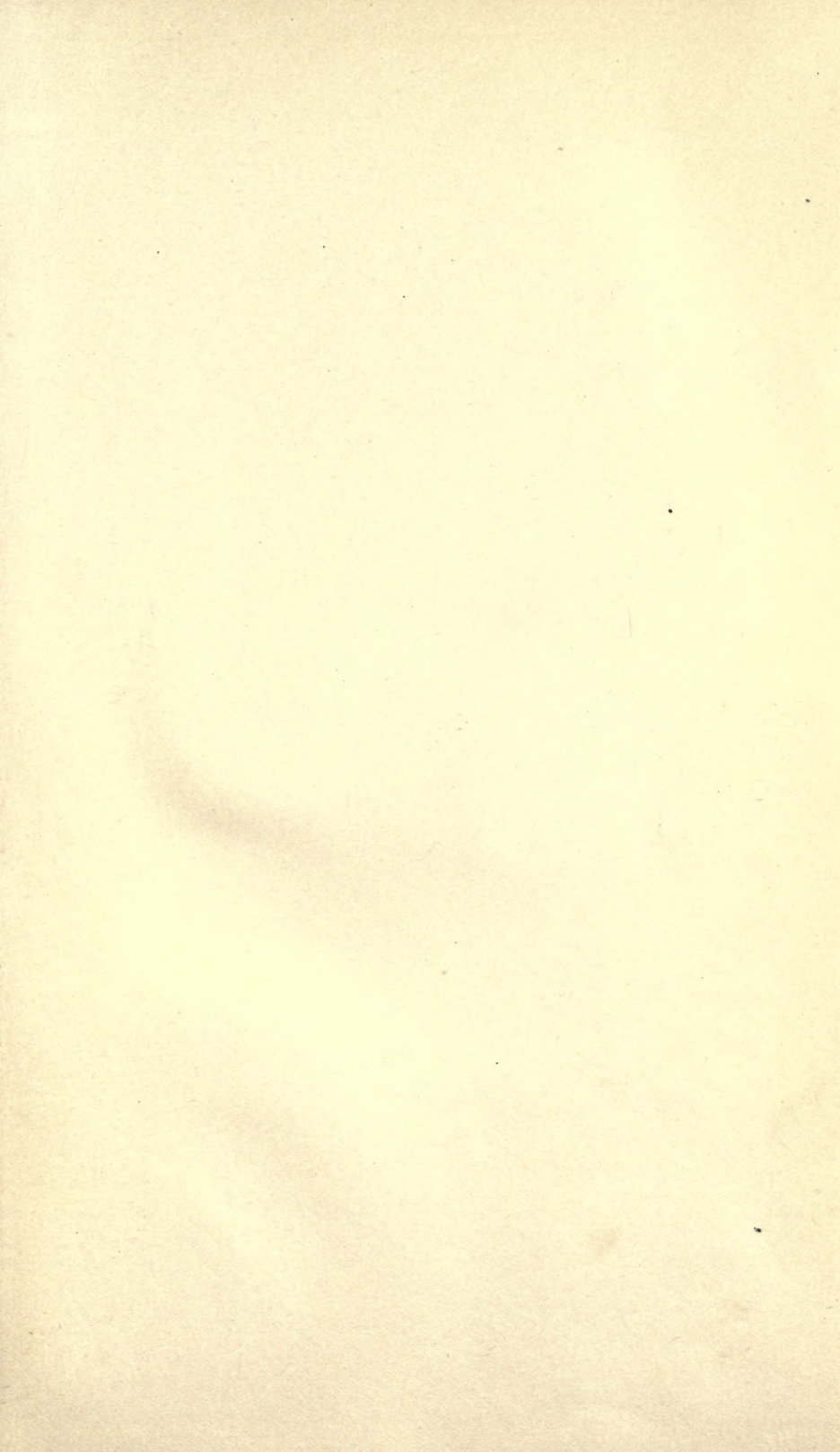
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CHEMICAL PATHOLOGY

BEING A DISCUSSION OF GENERAL PATH-
OLOGY FROM THE STANDPOINT OF
THE CHEMICAL PROCESSES INVOLVED

BY

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TO

Ludvig Mektoen

THIS BOOK IS RESPECTFULLY DEDICATED, AS A

SLIGHT TOKEN OF THE GRATITUDE AND

ESTEEM OF HIS PUPIL

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PREFACE

DURING the past score of years the subject of biological chemistry has attracted the attention and labors of a constantly increasing number of investigators, many of whom have, for one reason or another, been interested in pathological conditions. Sometimes the physiologist has sought for light on his problems in the evidence afforded by related pathological conditions. Frequently clinicians have studied the metabolic changes and the composition of the products of disease processes. Relatively seldom, unfortunately, has the pathologist attacked his problems by chemical methods. From the above and other sources have come scattered fragments of information concerning the chemical changes that occur in pathological phenomena. Only when bearing upon conditions such as gout and diabetes, which concern alike the physiologist, the clinician, and the pathologist, have the fragments been moulded together into a homogeneous whole. For the most part they still remain isolated, uncorrelated, frequently unconfirmed items of information, scattered through medical, chemical, physiological, and physical literature.

It has been the aim of the writer to collect these scattered fragments as completely as possible, and to use them as a basis for a consideration of General Pathology from the standpoint of the chemical processes which occur in pathological conditions. Owing to the diffusely scattered condition of the literature on which this work is based, it cannot be claimed that all of the many contributions from which useful information might be obtained have been noticed ; but it is hoped that a sufficiently thorough collection of material has been made to afford a fair basis for a consideration of "Chemical Pathology." The time seems ripe for an effort of this nature. Within the past few years great and encouraging advances have been made in biological chemistry, which in many instances seem to throw light upon pathological processes. In medicine, the use of chemical methods in the study of clinical manifestations has become more general, and has yielded valuable information. Pathologists have come to feel that the opportunities for the acquire-

ment of knowledge by means of morphological studies have become reduced to a minimum, while the fields of pathological physiology and chemistry lie still almost unexplored. The development of research upon the subject of natural and acquired immunity has presented innumerable problems, all of which are essentially chemical. And perhaps most important of all is the general awakening of an appreciation of the importance of physiological chemistry to medical science, which has led to the introduction of laboratory courses on this subject in every medical school worthy of the name.

A book on Chemical Pathology should, therefore, seek to supply information to a varied group of readers. It should furnish collateral reading to the student who for the first time goes over the subject of General Pathology, which his text-books usually consider chiefly from the morphological standpoint. It should exploit to the graduate in medicine the advances that are being made along lines that are of fundamental importance to clinical medicine. It should serve for the investigator in biological chemistry or in pathology as a source of information concerning the ground upon which the two subjects overlap—the “Grenzgebiete” of Pathology and Physiological Chemistry. And, above all, it should afford a guide to the sources of our knowledge of these subjects, since nothing but direct familiarity with the original reports of the investigators themselves can give the student an impersonal view of the actual status of the questions under consideration. On account of this multiplicity of the objects in view, it has often been necessary to consider certain topics from more than one standpoint; which explains, perhaps, certain apparent irregularities in the style and manner of treatment.

It has been assumed that the reader has at least an elementary knowledge of organic and physiological chemistry. For the benefit of those whose studies in these subjects date back some years, it has seemed advisable to include in an introductory chapter an epitome of the more modern views concerning the chemistry of the proteid molecule, the composition of the animal cell, and the principles of physical chemistry, in as far as they apply to biological problems. The general consideration of “Enzymes” in Chapter II is written with a similar object. In discussing these fundamental topics it has seemed advisable to omit detailed references to the numerous original sources,—these may be found quoted in the special text-books cited in the foot-notes; but in presenting the more distinctly pathological topics the attempt has been made to render all the important

literature available to the reader and investigator. To economize space, a complete bibliography has not been inserted when this exists already collected in some readily accessible review or original article; hence the references cited in the foot-notes will generally be found to include only the more recent publications. These references have been so selected, however, that they will be found to furnish bibliographical matter sufficient to lead the investigator to all the important literature on the topics covered in this book. As to those subjects (such as gout, diabetes, and gastrointestinal putrefaction) which, because of their great practical clinical interest, have already been discussed in available monographs at greater length than the scope of this work would permit, it has seemed appropriate merely to summarize the most recent views and advances, referring the reader to the special treatises for the general and historical discussions.

It is with the greatest pleasure that I acknowledge my indebtedness to many colleagues in the University of Chicago, who have kindly read the sections of my manuscript that touch upon their own special fields, and whose criticism and advice have been of the greatest assistance; their number alone prevents my thanking them by name. Most particularly, however, must I express my debt to my former instructor, Professor Lafayette B. Mendel, of Yale University, whose kindly criticism and suggestions have been of inestimable value. For constant assistance in the preparation of the manuscript, and for the revision of the bibliography, I am indebted to my wife.

H. G. W.

CHICAGO, January, 1907.

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CHEMICAL PATHOLOGY

WELLS

CHEMICAL PATHOLOGY

CHAPTER I

INTRODUCTION

THE CHEMISTRY AND PHYSICS OF THE CELL

SINCE Virchow founded modern pathology the unit of all anatomical considerations of disease has been the cell, and in physiology the same unit has been found equally useful. When either physiological or pathological processes are studied from a chemical standpoint, the cell is still found occupying nearly as fundamental a position, for we can seldom go back to molecules and atoms in investigating biological problems. Although we know that within each cell are many different chemical substances, and that numerous different enzymes and other agencies are exerting their influence upon them, yet we find that the reactions are all profoundly affected by the environment in which they occur, and it is the structure of the cell that determines the environment of its chemical constituents. All chemical reactions are modified by physical influences, and an enzyme may have quite a different effect upon a substance when it acts in a test-tube from what it will have when in a living cell, whose structure permits the diffusion of one substance while preventing that of another, and where countless other substances and enzymes may participate in the changes. The cell is the structural unit of the living organism, and as by its physical properties it modifies chemical processes, so it becomes practically the unit in physiological and pathological chemistry. All consideration of the chemistry of disease must thus refer back to the chemistry and physics of the normal cell, and on this account a brief résumé of these subjects may serve as a fitting introduction to the strictly pathological matters to follow.¹

¹ Of necessity, only so much of the very extensive literature on cell structure and cell chemistry can be considered as will have direct bearing upon the subject matter to follow, referring the reader for more detailed information to such works as Wilson's "The Cell in Development and Inheritance"; Mann's "Physiological Histology"; Hammarsten's "Physiological Chemistry"; Abderhalden's "Lehrbuch der physiologischen Chemie"; Gurwitsch's "Morphologie und Biologie der Zelle"; Höber's "Physikalische Chemie der Zelle und der Gewebe"; Hamburger's "Osmotischer Druck und Ionenlehre"; Loeb's "Dynamics of Living Matter", for general discussion, and to the most important monographs for treatment of special points.

As applied to the animal tissues, the term "cell" is entirely a misnomer, for it describes accurately only such forms of "cells" as are found in plants, in which the prominent feature is the limiting wall, forming a cell to enclose a fluid content. In most instances the "cell" answers better to the definition, "a mass of protoplasm"; but usage makes language, and no possible confusion can arise from the prevailing, universal use of the original term, except, perhaps, that the term is prone to carry with it the thought of the walls of the cell being much more prominent than they really are. This is not so unfortunate a result, perhaps, for, as we shall see later, the limiting surfaces of the cell, even when too thin to be readily demonstrable, play a much more important part in cell chemistry than their appearance indicates.

The morphological division of the cell into cell wall, cytoplasm, nucleus, and nucleolus can hardly be followed out chemically, for if we surmount to some extent the difficulties in the way of studying the different portions separately, we find that the differences between them are rather quantitative than qualitative. And, furthermore, however different the cells of one organ or tissue may appear from those of another organ or tissue under the microscope, when analyzed by the chemical methods at present at our disposal we find the differences very slight indeed. Certain substances are found in every living cell, and in quantities usually not greatly dissimilar; hence they are assumed to be the most important constituents of protoplasm, and are sometimes called the *primary* constituents of the cell. Many other *secondary* constituents may also be present, some of which are so nearly universal that we are not sure but that better methods would show them to be constant and primary cell components; such are fat and glycogen. Others are characteristics of certain cells, such as melanin and keratin, or specific products of cell metabolism, such as mucin and the specific enzymes. The great histological and chemical differences existing between different tissues depend often on these secondary products, as in fat tissue and squamous epithelium; or upon the intercellular substance, as with connective tissue, cartilage, bone, etc., which may be looked upon as products of cell activity.

Protoplasm, as the term is generally used, includes the various primary constituents with the fluids permeating or dissolving them, but does not include the more conspicuous secondary constituents, such as fat droplets, pigment granules, etc., nor the cell membrane when such exists. Evidently it is a very indefinite term, to be avoided as much as possible, par-

ticularly because of the confusion as to whether it includes the nucleus or not, different authors differing in this respect in their usage of the word.

CHEMISTRY OF THE ESSENTIAL CELL CONSTITUENTS

To enumerate the primary or essential constituents of the cell absolutely is not possible, for the rapid advances in chemistry may alter all classifications without warning, but practically they may be grouped under the headings of proteids, lipoids, salts, and water, and no attempt will be made to give here more than the most essential features concerning each.

PROTEIDS ¹

In the last few years we have obtained something approaching a scientific understanding of the chemical nature of this great group of the most highly complex bodies known to chemistry, although we are still far from a position where it can be positively said just how the various components of the molecule are united, or in exactly what proportion; and we are still farther, perhaps, from the point of synthesizing a full-fledged proteid molecule. But it is believed by many chemists that the problems regarding the underlying principles of the formation and structure of the giant proteid molecule are nearing solution. Our information has been obtained almost exclusively through studies of the products obtained by splitting up the proteids, for as yet little has been accomplished through synthesis. The names of Kossel and Emil Fischer are most prominently connected with this work. Proteids can be decomposed by the action upon them of acids or alkalies in various concentrations, by superheated steam, by digestive ferments, and by bacteria. The products obtained in these different ways are not all the same, for some substances may be formed by oxidation, reduction, decomposition, combination, or condensation of the various products of simple cleavage, and it is necessary to distinguish between the primary cleavage products (those which exist as radicals within the molecule) and the secondary products (those not existing preformed in the molecule but formed by transformation of the primary products). This can usually be done, and it is found that so far as the primary products are concerned, it makes little difference which method of cleavage (or *hydrolysis*, since in the splitting, water is combined with the organic substances) is used.

¹ For the complete literature of this subject see Mann's "Chemistry of the Proteids," New York, 1906.

At first the proteids split up into compounds still possessing many of the features of the typical proteid molecule, such as albumoses and peptones, and these bodies are then further resolved into simple substances, which are not aggregates of several smaller molecules as are the proteids, and which can be obtained in pure crystalline form. No matter which method is used we find the process going through these stages, and, as before mentioned, the primary crystalline products obtained are practically the same quantitatively as well as qualitatively. Some methods, *e. g.*, bacterial decomposition, however, lead in the end to more profound or different decomposition of the cleavage products into secondary substances. The similarity of the results obtained in these different ways indicates that there are definite lines of cleavage in the proteid molecule along which separation takes place, independent of the nature of the agency at work, and that the substances obtained represent, as the Germans figuratively say, the "building stones" of the entire molecule. A large number of such elementary constituents have already been isolated and identified with certainty, although there is no doubt that there remain others still undiscovered. The best known of these are the following:

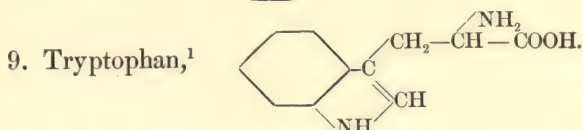
1. Glycocoll, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{CH}_2 - \text{COOH} \end{array}$.
2. Alanin, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{CH}_3 - \text{CH} - \text{COOH} \end{array}$.
3. Amino-valerianic acid, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ (\text{CH}_3)_2 = \text{CH} - \text{CH} - \text{COOH} \end{array}$.
4. Leucin, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ (\text{CH}_3)_2 = \text{CH} - \text{CH}_2 - \text{CH} - \text{COOH} \end{array}$.

These four bodies are all simple amino-acids of the fatty acid series, and represent typical members of the series as far as the hexane derivatives.

5. Aspartic acid, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{HOOC} - \text{CH}_2 - \text{CH} - \text{COOH} \end{array}$.
6. Glutaminic acid, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{HOOC} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{COOH} \end{array}$.

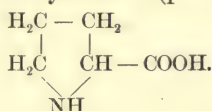
These two dibasic acids are also closely related to the monoatomic acids, as can be seen from their structural formulæ.

7. Phenyl-alanin, $\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{C}_6\text{H}_5 - \text{CH}_2 - \text{CH} - \text{COOH} \end{array}$.

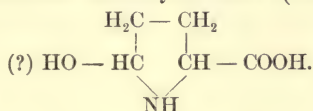


These three substances represent the aromatic constituents of the proteid molecule, and differ from the simpler amino-acids merely in the presence of the benzene ring.

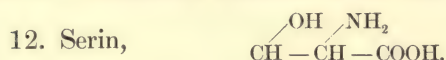
10. α -pyrrolidin carboxylic acid (prolin),



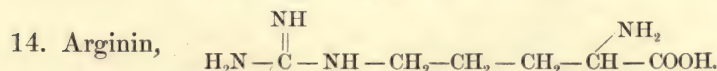
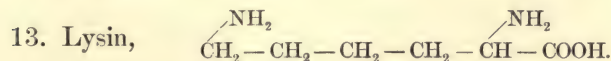
11. Oxy- α -pyrrolidin carboxylic acid² (oxy-prolin),



Both of these substances lie under the suspicion of being formed from some other amino-acid through re-arrangement within the molecule during the process of cleavage, but this idea has not been positively established, and they are among the most constantly obtained of the cleavage products.

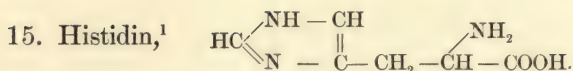


This substance, together with oxy- α -pyrrolidin carboxylic acid, is important as being an oxy-acid, which brings the proteid molecule into close relation with the carbohydrates. Amino-compounds even more closely related to the carbohydrates have occasionally been isolated (glucosamin) and it is possible that they are frequently present in proteids.

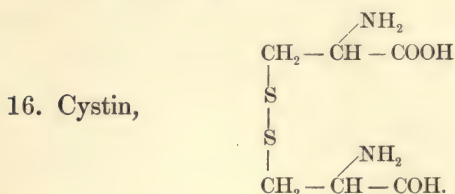


¹The exact structure of tryptophan has not been finally determined; the above formula, that of Ellinger, seems to be most probably the correct one.

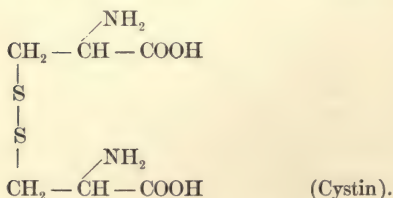
²The position of the OH group in oxy-prolin has not yet been finally determined



The members of this important group differ from all the bodies previously described in having more than one NH_2 radical. Kossel termed them the *hexone bases* because each has six carbon atoms, but the more descriptive term, *diamino-acids*, is now more generally used. On account of their wide occurrence, (no proteid has yet been found free from arginin) their prominent part in the formation of the so-called "simplest proteids" (the *protamins*), they have been held by some to form the real nucleus of the proteid molecule.



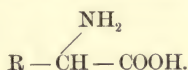
Apparently the sulphur in the proteids exists chiefly, if not solely, in this form. Cystin is closely related to the sulphur-free amino-acids, as can be seen by comparing the following formulæ :



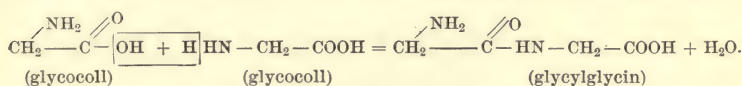
If we consider the composition of these substances, we notice that there is one important point that they all have in common : *each one is an acid, which has a NH_2 group substituted for a hydrogen atom on the carbon nearest the acid radical* (the α -

¹The structure of histidin is not finally determined. The formula given, that of Pauly, is believed to be correct.

position). It makes no difference what the rest of the radicals are, whether they are simple chains (leucin), or members of the cyclic or aromatic series (tyrosin), or sulphur-containing bodies (cystin), without exception this relation of a NH_2 group to an acid radical is constant, as in this formula :



Through this arrangement every one of the constituents of the proteid molecule is provided with a group with a strong basic character and a group with a strong acid character, and hence it is possible for them to unite with one another in indefinite numbers, and, because of the great variety of individuals, in practically an infinite number of combinations. It is believed that it is in just this way that the proteid molecule is built up. By artificially uniting various cleavage products Emil Fischer has succeeded in producing large molecules made up of several amino-acid radicals (called by him "polypeptids")¹ which show some of the characteristics of the peptones, and this is the nearest that investigators have yet come to synthesizing a proteid molecule. The union is accomplished by the splitting off of water, corresponding to the addition of water that occurs when the proteid molecule undergoes cleavage. It may be illustrated by showing the formation of the simplest polypeptid, *glycylglycin*.



For these reasons it is believed that *the proteid molecule consists of great numbers of amino-acid groups, combined with one another through their basic and acid radicals*, and that the various proteids are different from one another because they contain different numbers or varieties of amino-acids. For example, the *globin* of hemoglobin yields no glycocoll on hydrolysis, while *gelatin* yields 16.5 per cent. On the other hand, gelatin is free from tyrosin. Some of the *protamins* (proteids obtained chiefly from spermatozoa) yield as high as 58 to 84 per cent. of arginin, while the simpler amino-acids with but one N (mono-amino-acids) are scanty, and most varieties are lacking.

It will be noticed that when two amino-acids unite, as seen in the formation of glycylglycin, an acid radical and a basic radical are still left free. In this may be seen the explanation

¹ Reviewed by Fischer, in Ber. deut. Chem. Gesell., 1906 (39), 530.

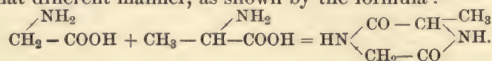
of the peculiar *amphoteric* nature of proteids. As long as these two groups are free the proteids can combine with either acids or bases, as they are well known to do, and hence they *react as either acids or bases under different conditions*.

It must not be imagined that the structure of the complete molecule is simply a long straight chain of amino-acids joined only in the same way as are the components of glycylglycin.¹ The existence of the diamino-acids, of the benzene rings, of hydroxyl groups, (as in serin or tyrosin), of ring compounds, (as pyrrolidin-carboxylic acid), of substances with two acid groups, (as glutaminic and aspartic acid), adds complications to the formation until it is impossible to estimate just how all the various building stones may be arranged. We must bear in mind the size of the proteid molecule, which Hofmeister has estimated (for serum albumin) as having a molecular weight of 10,166, and for hemoglobin the molecular weight has been estimated at 16,669. Within such a "giant molecule" there is room for variety almost beyond computation.

The Proteids of the Cell.—By physiological chemists proteids are classified into *simple proteids*, of which egg and serum albumin are types; and *compound proteids*, which are characterized by having some special non-proteid group which can be split off, leaving behind a characteristic proteid residue, *e. g.*, nucleo-proteids, glyco-proteids. As primary cell constituents the following varieties of proteids may be mentioned: albumin, globulin, nucleo-proteid, nucleo-albumin or phospho-proteid, and coagulated proteids. At one time it was thought that cytoplasm consisted chiefly of albumin, like white of egg, but we now know that this forms but a small part of the cell proteids, often occurring only as traces. It is held by some that true albumin occurs only as a building or intermediate cleavage product of the more complicated forms of cellular proteids, and is itself of relatively slight importance in cell life, not participating in chemical changes except as a food-stuff.

Albumins are characterized chiefly by their greater solubility in water, and in being less easily precipitated than most proteids. They seem to be a fundamental type of proteids. The three forms of albumin that have been described in animal tissues or products are egg-albumin, lactalbumin of milk, and serum

¹ Fisher and Abderhalden (Ber. deut. Chem. Gesell., 1906 (39), 752) have described a polypeptid in which the union of the amino-acids is accomplished in a somewhat different manner, as shown by the formula:



albumin ; probably cell albumin is most closely related to the last, and what has been described as cell albumin is perhaps in many cases but serum albumin that has been imperfectly removed.

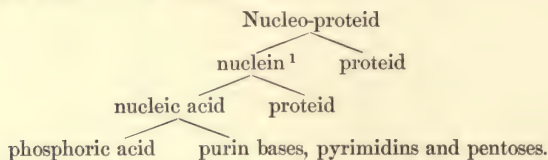
Globulins also occur in all cells, but in small amounts in most animal cells except the muscles, whose chief proteids belong to this or a closely related group. The globulins are quite similar to the albumins, so that there is really no sharp line between the two groups. Their insolubility in water separates them from albumins, and their solubility in dilute neutral salt solutions from the more complex proteids. An important feature of the globulins is the low temperature at which they coagulate—some so low that Halliburton¹ believes it possible that they may be coagulated within the cells during high fevers.

Hammarsten has long maintained that simple proteids form a relatively insignificant part of the cytoplasm, in opposition to the once-prevalent view that the nucleo-proteids were limited to the nucleus, and that the cytoplasm was chiefly albumin and globulin. The general trend of opinion as influenced by the results of researches has been favorable to his contentions, and we shall probably not be far wrong in accepting his statement that—"The chief mass of the protein substances of the cells does not consist of proteids in the ordinary sense, but consists of more complex phosphorized bodies, and that the globulins and albumins are to be considered as nutritive materials for the cells or as destructive products in the chemical transformation of the protoplasm."

Nucleo-proteids are probably the most important constituents of the cell, both in quantity and in relation to cell activity. The enzymes seem to be nucleo-proteids, or at least they are intimately associated with them. (See further discussion under the subject of enzymes.) In structure the nucleo-proteids are very complex, as indicated by the different products yielded on hydrolytic cleavage of the molecule. Furthermore, there are many varieties, depending both upon the nature and proportions of the component parts. They may be described as consisting of two primary constituents—(1) nucleic acid and (2) a proteid body, in chemical combination with each other like a salt. In the chromatin structures of the nucleus the proportion of proteid in the nucleo-proteid is small, so that these bodies have a strongly acid character, as indicated by their affinity for basic

¹ Halliburton and Mott, *Archives of Neurology*, 1903 (2), 727 ; also see Halliburton's "Chemistry of Muscle and Nerve."

stains. In the cytoplasm, on the other hand, the nucleo-proteids have the acid quite saturated with proteids and hence are devoid of acid properties, which is also indicated by their lack of affinity for hematoxylin and other basic dyes. The term *nucleic acid* also covers a large group of substances, which are characterized, on the one hand, by their frequent occurrence bound with proteids, and, on the other hand, by their yielding phosphoric acid and purin bases, pyrimidins and pentoses on cleavage. Diagrammatically the manner of cleavage of the nucleo-proteids may be indicated as follows :



The enormous variety of nucleo-proteids that may possibly exist can be imagined when we consider that there exist several different sorts of purin bases, not all of which are found in any one nucleic acid, that the form of phosphoric acid present may vary, that the proteids are of different varieties, that the proportions of each ingredient is perhaps never twice the same, and furthermore that many nucleo-proteids contain carbohydrate groups. The possible combinations of these ingredients is little short of infinite and it may well be that we have here a partial explanation of the innumerable varieties of living organisms.²

In the cell the nucleo-proteids probably exist partly as solid structures, *e. g.*, the chromatin framework of the nucleus, and partly dissolved in the plasma. An interesting phenomenon is the alteration in the chromatin nucleo-proteids during cell division, when they seem to lose part of the combined proteid and approach more nearly pure nucleic acid—just as inorganic salts occur with the acids and bases saturating each other more or less incompletely, *e. g.*, mono-, di-, and tribasic phosphates. In this we have a chemical explanation of the intensity of the staining of dividing nuclei by basic dyes.

Nucleo-proteids combined with carbohydrates, *nucleo-glucoproteids*, are probably important and perhaps constant cell constituents. It is of interest to note that the carbohydrate is often not one of the ordinary hexoses, such as glucose, but one of the more uncommon *pentoses*.

¹ Probably nuclein should be considered as merely one variety of nucleo-proteid, with less proteid than the other varieties.

² The chemistry of the nucleo-proteids is also discussed in the chapter on Uric Acid Metabolism and Gout, Chap. XXI.

Nucleo-albumins (or *phospho-proteids*), by an unfortunate similarity of name, are often confused with nucleo-proteids by non-chemical writers, a difficulty increased by an actual resemblance to the extent that they also yield phosphoric acid, and are somewhat similar in solubility and digestibility. They are essentially different, however, in that they *do not yield nucleic acid* or purin bases on cleavage. Probably members of this group are also constant components of cells.

Glycoproteids (or *gluco-proteids*) and *phospho-glycoproteids* are also believed to occur frequently or constantly in protoplasm. They are compounds of proteids with a sugar or sugar-like group, which probably usually contains nitrogen, thus differing from the ordinary hexoses and pentoses.

Insoluble proteids, or bodies resembling the coagulated proteids in their lack of solubility in various fluids, are left behind after the other proteids have been extracted from the cells. Their significance is not known: whether to a large extent artificially produced or whether a normal structural element of the cell.

FATS AND LIPOIDS

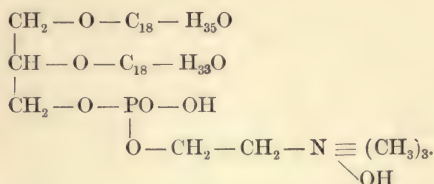
Ordinary fats occur in nearly all cells, and probably in all, but their demonstration is not readily possible. The microscopic appearance of a cell, even when special stains for fat are used, gives no correct idea of the amount of fat actually present. Thus normal kidneys contain 15 to 18 per cent. of fat in their dry substance, but none of this can be detected with the microscope. A kidney which seems microscopically the site of marked fatty degeneration may show no more fat when examined chemically than a normal kidney, which in section appears to be quite free from fat. This is because some of the intracellular fat is bound chemically with the proteids, and when so bound it cannot be seen, nor can it be stained by the dyes used for that purpose; only when degenerative changes of certain kinds have liberated it from combination does it become visible and stainable (Rosenfeld). Whether the intracellular fat has any function other than that of serving as a food-stuff is not known, but there can be no question of the importance of the phosphorized fat, lecithin.

Lecithin is a primary cell-constituent, and is probably important both in metabolism and physically. Hammarsten regards it as concerned in the building up of the nucleus. As will be shown later, many of the most essential physical properties of the living cell depend upon the presence in it of lipoids, of which lecithin is apparently the chief. Of the ether-soluble

substances in the heart, for example, 60 to 70 per cent. is lecithin, which constitutes about 8 per cent. of the dry weight of the myocardium.

There are several varieties of lecithin, depending upon the fatty acid radical they contain, and for the group Koch has proposed the name of *lecithans*.

The structural formula of one lecithin, stearyloleyyl lecithin, is as follows :



It differs from ordinary fats, therefore, in having two special groups, one the phosphoric acid, the other the cholin radical, which last seems to be of no little importance in pathological processes. In its physical properties it is quite similar to the ordinary fats, although it forms even finer emulsions in water, which are practically colloidal solutions (W. Koch).

Cephalin, a closely related body differing in having but one methyl group, is also probably as widely spread in the tissues as lecithin, according to Koch and Woods.¹

Cholesterin, which is another lipid, is nearly as universally present as lecithin.² There are probably several varieties of cholesterin, which exist both free and in combination with fatty acids, for cholesterin is an alcohol and not at all similar to the fats chemically, although very similar physically. The empirical formula is $\text{C}_{27}\text{H}_{44}\text{O}$ (or $\text{C}_{27}\text{H}_{46}\text{O}$) and it is possibly related to the terpenes. It seems to be quite inert chemically, and therefore is probably important only because of its effect on the physical properties of the cells. By some it is considered to be a decomposition or cleavage product of the proteids, which is in abundance with its abundance in masses of old necrotic tissue, *e. g.*, atheromatous masses, old infarcts, and old exudates.

Protagon, which name probably covers a group of nitrogenous, phosphorized bodies, (Gies³), occurs in many or all cells, but especially in the nervous tissues. The properties of protagon are in general similar to the other lipoids, but its exact

¹ Jour. Biol. Chem., 1905 (1), 203.

² Recent literature given by Abderhalden and Le Count, Zeit. exp. Path. u. Pharm., 1905 (2), 199.

³ Jour. Biol. Chem., 1905 (1), 59.

composition is evidently too uncertain to permit of surmises as to its special purpose.

Jecorin, which is generally considered as a combination of lecithin and glucose, is probably also not a definite compound, according to the most recent observations.¹

CARBOHYDRATES

The third great class of food-stuffs, the carbohydrates, is represented in the cell by *pentoses* and *hexoses* combined with proteids and with lipoids, and also by *glycogen*, which exists free. Glycogen is a rather difficult substance to isolate, and, therefore, although it is not found in all cells by our present methods, yet it may well be that it is a constant constituent of the protoplasm. There is no evidence, however, that it is anything more than a source of heat and energy to the cell. Its properties and occurrence will be considered more fully in the discussion of glycogenic infiltration. Since glycogen is formed from dextrose and is constantly breaking down into dextrose, it is probable that the latter is also constantly present in the cells.

INORGANIC SUBSTANCES

Up to this point the substances of the cytoplasm that have been discussed have all been organic compounds which do not naturally exist independent from living or once living cells, yet the inorganic substances of the protoplasm are also of vital importance. As Mann says, "so-called pure ash-free proteids are chemically inert, and, in the true sense of the word, dead bodies. What puts life into them is the presence of electrolytes." The various salts of potassium, sodium, calcium, magnesium, and iron which all cells contain do not exist merely dissolved in the water of the cell, but in part they are combined with the organic constituents of the protoplasm. They are not combined as simple additions of the salts to the proteids; but *ions*, both anions and cations are united in chemical combination to the large proteid molecule (ion-proteids). Possibly the proteids participate in vital chemical processes only as ion compounds with inorganic elements. It is extremely difficult, indeed almost impossible, to secure proteids entirely free from inorganic substances (ash-free proteids). The fact that the inorganic substances are held in the cells chemically rather than by simple diffusion into them from the surrounding fluids is shown by the great difference in the proportions of various salts

¹ Meinertz, Zeit. physiol. Chem., 1905 (46), 376; Siegfried and Marx, *Ibid*, 1906 (46), 492.

in the cells and in the extra-cellular fluids. Thus potassium is nearly always much more abundant in the cells than in the tissue fluids, while sodium is more abundant in the fluids. Phosphoric acid is also more abundant in the cells, and chlorin in the plasma. In cells iron seems to exist chiefly in combination with the nucleo-proteids. These matters will be taken up in greater detail in considering the physical chemistry of the cell.

THE PHYSICAL CHEMISTRY OF THE CELL AND ITS CONSTITUENTS

From the standpoint of physical chemistry the cell consists of a collection of colloids and crystalloids, electrolytes and non-electrolytes, dissolved in water, in lipoids, and in each other, surrounded by a semipermeable membrane, and perhaps subdivided by similar membranes. Physical chemical processes, as we shall see later, play an all-important part in the life phenomena of the cell, and therefore some space may profitably be occupied in explaining the nature of these changes and of the substances that participate in them.

CRYSTALLOIDS AND THEIR PROPERTIES

Crystalloids, or substances that tend under favorable conditions to form crystals, and which diffuse readily through most diffusion membranes, form a relatively small part of the total mass of the cell, but they are fully as essential as the colloids. The chief representatives of this group that are found usually or constantly in the cell are the inorganic salts, sugar, and the innumerable decomposition products of the proteids, including particularly urea, creatin, purin bases, amino-acids, etc. Most of these are by no means so characteristic of living things as are the colloids, sometimes occurring quite independently of a cellular origin, which the proteids never do. The inorganic salts in particular seem quite foreign to living processes, and as they enter and leave the body practically unchanged they are evidently not a source of energy through chemical change. Their importance to the cell lies almost entirely in their physical or physico-chemical properties. The organic crystalloids, although of nutritional value, also have physical properties in some respects similar to those of the inorganic crystalloids, and therefore to this extent they exert similar influences, but the essential difference between the organic and the inorganic crystalloids is that all the latter are electrolytes, while many of the organic crystalloids that occur in cells are non-electrolytes. The importance of this distinction lies not in the utility

or non-utility of these substances as conductors of electrical currents in the ordinary sense, but rather on the existence of those properties which determine their conductive ability. Electrical conductivity is an index of ionization, and upon ionization depends the chief influence of the electrolytes upon vital activities.

Electrolytes and Non-electrolytes. Ionization.—

If we attempt to pass a current of electricity through water, we find that it meets a great resistance, and the purer the water, the greater the resistance. In water as pure as can possibly be obtained the resistance of a layer only one millimeter thick has been found equal to that of a copper wire of equal cross-section long enough to reach around the earth one thousand times.¹ The addition of the slightest quantity of salts, acids, or alkalis increases the conductivity enormously, and in any considerable amounts they make the solution an excellent conductor of an electric current. On the other hand, by the addition of sugar or alcohol to the water, the conductivity is increased very little or not at all. What differences exist between the soluble substances that do increase the conductivity of the solution and those that do not?

If we dissolve in water in a platinum dish a small quantity of an electrolyte, say copper sulphate, and pass a current of electricity through it, using the platinum dish as the negative electrode and inserting the positive electrode in the solution, it will be found after a short time that there is no longer a blue solution of copper sulphate in the dish, but rather that the lining of the dish, where it is under the liquid, has become red from the deposition of a thin layer of copper. If we reverse the current, it will be found that the copper leaves the surface of the dish to collect upon the electrode inserted in the water, which is now the negative pole. This experiment illustrates the ability of the copper to wander from one electrode to another, and it is by this wandering that the electricity is carried by the copper particles. The copper sulphate is dissociated into its two parts: copper, carrying a positive charge which goes to the negative pole or cathode, and is therefore called the positive ion, or *cation*; SO_4 , carrying a negative charge, wanders to the anode, and is therefore called the negative ion or *anion*. The individual particles which carry the charges are designated as *ions*. It will be noted that the particles may or may not be single atoms; in the case of copper sulphate the cations (copper) are atoms, but the anion, SO_4 , is formed by several

¹ Kohlrausch and Heydweiller, quoted by Cohen.

atoms grouped together. Sometimes the ion consists of a great number of atoms, as when such a molecule as stearic acid dissociates we have ions of hydrogen and ions of $C_{18}H_{35}O_2$. In general, if the ion is very large, its movement is relatively slow, and it shows less pronounced chemical properties.

Now, although the migration of the ions to the electrodes has been known for a very long time, the important fact that the separation of a substance into its ions is not brought about primarily by the electric current was ascertained later. It is not the passage of the current that splits up the electrolyte, but rather it is because the substance has already been split by the solvent into its ions that it conducts the current. When we dissolve an electrolyte, say sodium chloride, in water, many of the molecules split into the cation Na, and the anion Cl. If the solution is very dilute, the dissociation may be complete, and we have no molecules of NaCl in our solution at all, but merely the two sorts of ions in rapid motion. If the solution is more concentrated, a larger proportion remains undissociated, although the total number of ions may be much greater. What the electric current does in passing through such a solution is to cause a migration of ions toward the respective poles, where they accumulate; as a result, the solution between the poles contains fewer ions than it should and the molecules undergo continuous dissociation until they have finally disappeared, for the ions are all collected about the poles as fast as formed, and finally the solution becomes free from both molecules and ions except in the vicinity of the poles. So complete is this migration that the most accurate method of quantitative estimation of many metals, such as copper, is this electrolytic method, by which we can cause all the copper ions to become attached to the inner surface of a weighed platinum dish, and after washing away the solution and drying we can determine accurately the amount of copper that has been attached to the dish.

It is the act of solution, then, and not the electric current, that causes ionization, and so every solution of an electrolyte, such as a physiological salt solution, or sea water, or urine, or any secretion of the body, contains a greater or less number of free ions. In $\frac{n}{10}$ salt solution, which is nearly the same concentration as physiological salt solution (its strength is 0.58 per cent.), the amount of dissociation is so great that 84 per cent. of the molecules of NaCl have been changed into the ionic form and but 16 per cent. remain as molecules at room temperature. Evidently, since it is the solvent that causes the dissociation, the nature of the solvent will make a great difference

in the amount of dissociation. Water is the best known medium for causing dissociation, except possibly peroxide of hydrogen, while chloroform and alcohol are relatively very weak in this respect. The amount of dissociation is also increased by raising the temperature.

The importance of this process of dissociation or ionization lies in the fact that with most substances no chemical reaction can occur while the substance is in the non-ionized state. The chemical properties of ionizable substances are produced largely by the ions they liberate on dissociation. Acids owe their character to the hydrogen ion, alkalies owe theirs to the hydroxyl ion. We can appreciate the difference between the ions and the same substance in the non-ionized form if we consider the chemical inertness of hydrogen gas, as compared with a solution of acid which owes its powerful effects to hydrogen ions. Perfectly dry sulphuric acid is absolutely free from the acid properties that characterize it when it contains a little water, because it is not ionized when dry. It is for the same reason that we can have two substances together in a dry condition without reaction, that would immediately react if moist. It is by means of the electrical charges of the ions that chemical reactions occur, and hence ions must be present to have reactions. As a consequence, the physiological effects of electrolytes are due to their ionic condition, and through the ions that are present in the cell many of its various chemical processes are brought about. Not all substances ionize with the same readiness, which causes a great difference in their properties. The reason that acetic acid is a weaker acid than hydrochloric acid is that it does not ionize to such an extent, and so a corresponding quantity does not introduce as large a number of hydrogen ions into a solution. Larger molecules, as a rule, ionize less than smaller ones of similar nature, *e. g.*, stearic acid ionizes less than acetic acid and therefore is a weaker acid. Likewise the properties of a substance which depend upon its ions will be less marked when it is in a solvent that produces little ionization. For example, bichloride of mercury owes its antiseptic properties to the Hg ions that it sets free when in solution. It is well known that solutions of mercury, and for that matter most other antiseptics, are much less actively germicidal in alcohol than when in water, because their ionization is less in alcohol; and the germicidal properties decrease as the proportion of alcohol increases, until the germicidal effect of the mixture is no greater than that of alcohol alone in the same strength.

If we had no electrolytes in the cell, electric charges could

not be carried about in it, and hence chemical reactions could not occur. It is this fact that makes the inorganic salts of such vital importance to the cell life. To repeat Mann's words, it is the electrolytes that put life into the proteids. Water itself is almost absolutely nondissociated, and proteids so little that for some time it was doubted if they really did ionize. Probably all soluble substances do dissociate to a certain minimal degree, but it is so slight for most of the constituents of the cell except the inorganic salts (the organic acids and alkalies, and a few dissociable organic products of proteid metabolism, occur in such insignificant amounts as to be almost negligible) that without them there would be little chemical activity possible, and hence life would be absent or at a very low ebb indeed. As before mentioned, the inorganic salts probably exist in the cell not only as salts, but also, and perhaps chiefly, as ions and ionic compounds with the cell proteids. For the most part it seems to be the cations that play the chief rôle in forming ion-proteid compounds, although undoubtedly the anions do combine with the proteids also, and in some instances they exert very characteristic and important effects; *e. g.*, the differences between the effects of chlorides, bromides, and iodides, or of CNH as compared with HCl, both of which liberate the same cation and differ only in their anions.

Many applications of the facts and theories of ionization have been made in physiology, as, for example, the observation of Kahlenberg and True that taste is produced by ions rather than by whole molecules; of Loeb, on the effects of ions upon the taking up of water by the cells and tissues, their effects upon muscular contractions, and upon cell multiplication and fertilization; of Mathews, upon the transmission of nervous impulses; of Hardy, upon the effects of ions on coagulation and precipitation of colloids. A few applications have also been made in pathology, especially the relation of ions to edema, to diuresis and glycosuria, and also to problems of immunity. No attempt will be made here to go further into the observations and theories concerning ionization or its rôle in physiology, but for more extensive information as well as for the complete bibliography the works mentioned below may be referred to.¹

¹ "Physical Chemistry for Physicians and Biologists," Cohen. American translation by M. H. Fischer, 1903; New York. "Physikalische Chemie der Zelle und der Gewebe," Höber, Leipzig, 1902. "Osmotische Druck und Ionenlehre in den medicinischen Wissenschaften," Hamburger, Wiesbaden, 1902. "Studies in General Physiology," Loeb, University of Chicago Press, 1905. "Dynamics of Living Matter," Loeb, Columbia University Press, New York, 1906.

The applications in pathology will be brought out as the subject under discussion in subsequent chapters necessitates, and it is largely to facilitate the understanding of such references that this brief summary of the subject of ionization has been introduced. In the same spirit we take up the subjects of diffusion and osmosis.

Diffusion and Osmosis.—Although the non-electrolytes do not ionize to any considerable extent, and therefore are relatively inactive chemically, the crystalloidal non-electrolytes, of which sugar and urea are the two chief examples among the cell constituents, possess in common with the electrolytes the important property of diffusion. By this process the exchange of chemical substances between the blood and the cell is brought about, by it the chemical composition of the different parts of the cell and between different cells is equalized, and without it chemical change would be practically impossible. Diffusion occurs most simply between two solutions of unlike nature, or between a solution of a substance and the solvent alone, when placed in contact with one another. If we place in the bottom of a cylindrical vessel a solution of copper sulphate and above it some water, carefully avoiding mixing, it will be found after some time that the fluid has become equally blue throughout. This is brought about by the movement of the dissolved particles, which gradually carries them through the entire mass of fluid, and as their migration is against the force of gravity, they evidently accomplish work. This process is not dependent upon ionization, for a solution of cane-sugar or of urea will show the same diffusion. A solution of proteid or other colloid does so much more slowly, however, indeed quite imperceptibly.

If we were to introduce a piece of filter-paper between the water and the copper sulphate solution, the diffusion would go on the same, the pores of the paper permitting the passage of the molecules without hindrance. If, instead of filter-paper, there were introduced a sheet of some substance free from pores, then diffusion would be much more affected. If the septum was of such a nature that the substances in solution were insoluble in it (*e. g.*, glass), diffusion would of necessity stop; but if it were something in which the solvent or the solute was soluble, such as a gelatin plate, then these substances would dissolve in it, and diffusing through its substance escape into the fluid on the other side. The last example indicates the conditions afforded in the animal cell, and also in the usual laboratory diffusion experiments when the membrane is generally either an animal membrane or a parchment paper, both of

which are composed of colloids. Crystalloids are generally soluble in colloids and hence pass through such diffusion membranes; colloids dissolve but slightly in colloids, and hence they do not pass through a diffusion membrane readily, and are, therefore, but very slightly diffusible.

The process of diffusion, if uninterrupted, always continues until the solution is of exactly the same composition throughout. If on one side of the diffusion membrane there is a substance that passes through the membrane rapidly, and on the other a substance that passes through slowly or not at all, there will soon be an unequal condition on the two sides of the membrane, for the diffusible substance would accumulate in equal amounts on each side, while the non-diffusible would remain where it was. On one side there would then be more material exerting osmotic pressure than on the other, and if the membrane were flexible, it would bulge toward the opposite side. The pressure is due to the bombardment of the containing walls by molecules or ions of the substances in solution, and hence the more molecules and ions in a solution, the more pressure. When equal numbers of particles are on each side of the partition, the pressure is equalized. It is quite possible to have membranes permeable to one substance and not to another; such membranes are called *semipermeable*. Experimentally they are usually produced as follows: A cup or cylinder of porous clay, such as the cylinder of a Pasteur-Chamberland filter, is filled with a solution of some substance and placed in a solution of another substance, which, by reacting with the first where they meet in the wall of the cylinder, forms the proper sort of a precipitate—most frequently copper sulphate and potassium ferrocyanide are used, or gelatin and tannic acid. A thin film or membrane of the precipitate is formed in the wall, which is supported firmly by the clay, so that large pressures can be developed without destroying the membrane. If we now fill the cup with a solution of sugar or some other soluble crystalloid, its particles will bombard the walls of the cylinder in vain; they cannot pass through the semipermeable membrane. On the other hand, the water can pass through, and does so in an attempt to equalize the concentration on both sides of the membrane, and hence the volume of fluid in the cylinder increases. This it will do until the weight of the column of liquid in the cylinder balances the osmotic pressure, and in this way we can measure just how great the pressure is. The amount of pressure exerted by a substance in solution is thus learned to be very great; a 6 per cent. solution of cane-sugar produces a

pressure of 3075 millimeters of mercury at 14° (about sixty pounds to the square inch). To produce osmotic pressure it is not necessary that the membrane be absolutely impermeable to any of the substances—it may only be relatively less permeable for the solute than for the solvent. If, for example, we fill a parchment bag with concentrated sugar solution, tie up the top tightly and throw into water, it will swell up rapidly and eventually burst. But if the parchment is in the form of a tube, open at the top, and the lower end is placed in water, the amount of fluid inside the tube will increase at first, but eventually the sugar will diffuse out to such an extent that the solution is of the same concentration inside and outside of the tube, and the column of fluid will again become of equal height on both sides. These results indicate that the water passes through the membrane more rapidly than does the sugar, but that eventually the sugar can all pass through.

Exactly similar conditions exist in cells, particularly plant cells. The typical cell of plant tissues consists of a cellulose wall, lined internally by a layer of protoplasm which incloses a mass of aqueous solution, the cell sap, containing sugar and various other solutes. The cellulose wall is readily permeable by water and by most solutes, whereas the protoplasmic layer inside it behaves like a semipermeable membrane which permits water to pass through readily but hinders greatly the passage of most solutes; that it is somewhat permeable is attested by the fact that the cell sap contains solutes derived from the external fluids. As a result of this arrangement there is a constant tendency for the cavity of the cell to be distended by water and for the solutes within it to exert their considerable pressure upon the cell wall. Because of the strength of the cellulose layer the cell can withstand great pressures that would tear apart the tender protoplasmic layer that really determines the osmotic conditions, just as in the experimental membrane the clay cylinder supports the delicate precipitation membrane. It is the osmotic pressure that causes the rigidity or *turgor* of plant cells, and explains the ability of a tender green shoot to hold itself upright or horizontal in the air; and it is the force that enables growing roots to lift great stones or tear apart rocks in whose clefts they grow. If plant cells are placed in distilled water, the pressure may rise to such an extent that the cells burst, and it was through studying this phenomenon that Pfeffer worked out the basis of our present knowledge of osmotic pressure. If the cell is placed in a solution of greater concentration than its cell sap, the pressure outside will be

greater than that inside and the protoplasmic membrane will be forced away from the cellulose wall, while its central cavity shrinks and perhaps disappears entirely, the protoplasm forming a ball in the center. This is practically what occurs when a plant stem is cut and it "wilts"—the water is removed by evaporation, the osmotic pressure outside the cells becomes greater than that inside, and the water passes out. Likewise when a plant cell dies the turgor is lost because the membrane becomes permeable, and so pressure soon becomes the same on both sides of the cell wall.

In animal cells the wall is not so highly developed as in plants, nor is it backed up by a rigid material like cellulose; indeed, for many animal cells there is no well-defined wall and the protoplasm appears to be naked. Nevertheless the behavior of the animal cells indicates that they do possess what resembles a cell wall, in that they behave when in solutions as if they were surrounded by a diffusion membrane. The degree to which phenomena of this nature are shown varies with different cells; with red corpuscles, for example, the osmotic pressure influences are very marked, as shown by the wrinkling or crenation of the corpuscles when they are placed in fluids of higher concentration than the blood plasma, and by their swelling and disintegration with escape of the hemoglobin (*hemolysis*) when they are put into distilled water or solutions of less concentration than the plasma. Other tissue cells seem to undergo more or less alteration from changes in the osmotic pressure in the fluids surrounding them. The diffusion membrane that surrounds the cell is generally not well defined, and for most cells seems to be but a surface condensation of the protoplasm, perhaps formed through the effects of surface tension. The diffusion within the cell, however, seems to be so much more free than it is through the cell wall that it is probable that the surface layer of the cell is quite different from that of the rest of the cytoplasm. It seems probable that this surface diffusion membrane contains a large proportion of cell lipoids, *i. e.*, cholesterin and lecithin (for the red corpuscles this is practically certain); hence substances soluble in lipoids penetrate the cell readily, while to substances insoluble in lipoids the cell is nearly or quite impermeable (Overton). Probably the wall of the animal cell is not so nearly semipermeable as is that of the plant cell, for nowhere in the animal body do we get such turgor in the cells as we see in plant tissues. Lacking a cellulose wall, animal cells could not develop such an internal pressure without rupturing, and such a process of rupturing

(*plasmorrhaxis*, *plasmoptysis*) does not seem to be a normal occurrence in animal tissues. We shall be most nearly correct, probably, if we look upon the animal cell as possessing a delicate diffusion membrane at its surface, through which water passes more readily than do most crystalloids, and through which colloids pass almost not at all, but the exclusion of each of these types of substances is merely relative and not absolute.

Since osmotic pressure, exactly like gas pressure, is produced by the bombarding of the walls of the container by particles in the solution, the amount of pressure will vary in proportion to the number of particles present. With such substances as sugar and urea, the non-electrolytes, the moving particles seem to be molecules, and so a solution of sugar or urea will produce an osmotic pressure directly proportional to the number of molecules it contains. In the case of the electrolytes, however, the ions produce pressure as well as the molecules, and hence an electrolyte in solution will produce a relatively high osmotic pressure as compared with an equivalent solution of a non-electrolyte, since each molecule yields two or more ions. Colloids, however, exert so slight an osmotic pressure that it is difficult of detection; this probably depends on the great size and slight motility of their molecules. In the many and important osmotic processes of the animal organism, therefore, the colloids take no part except in helping to form the diffusion membrane, and in preventing the diffusion of one another. It is interesting to consider also that colloids under ordinary conditions do not greatly modify the diffusion of crystalloids through a solution containing both classes of matter. The fact that a cell is full of dissolved colloids does not seriously affect the osmotic properties of the intracellular crystalloids, provided it is not condensed in such a way as to form diffusion membranes. But as all the cleavage products of proteids after they have passed the peptone stage are crystalloids (*e. g.*, leucin, tyrosin, glycocoll, etc.), by decomposition of the intracellular proteids the osmotic pressure may be greatly raised. As long as the cell is living there can be no constancy in composition, for metabolic processes, by producing from proteids that have no osmotic pressure crystalloidal substances that do have osmotic pressure, cause intracellular osmotic conditions to be continually varying. As a result, streams of diffusing particles are moving about in every direction, setting up new chemical reactions and consequent new osmotic currents. The greater the difference in osmotic pressure between a cell and its

environs, and between the different parts of the same cell, the more powerful the osmotic effects, and as a result the greater the capacity for accomplishing work. The storing up of insoluble and indiffusible forms of substance, such as glycogen, fat, and proteids, is an important factor in maintaining inequalities in osmotic pressure, and in this way of increasing work capacity.¹

The relation of osmotic pressure and osmosis to physiological problems is only beginning to be studied. It is apparent that they must be of essential importance in absorption from the alimentary canal, in absorption and excretion between the cells and the blood stream, and in secretion by glandular organs; but it is also certain that they are no less important in all the less obvious chemical and physical processes of the cell. These matters will not be discussed here at length.² In pathological processes osmotic pressure may play an equally important rôle, and the facts discussed in the preceding paragraphs will be alluded to frequently in subsequent chapters.

COLLOIDS³

Since Graham in 1861 studied the differences between the substances that did or did not diffuse readily through animal or parchment membranes, soluble substances have been classified in the two main groups of *colloids* and *crystalloids*, which distinction Graham believed separated two entirely different classes

¹ J. Traube has developed a theory of osmosis, depending upon *surface tension* which appears to be of much importance (Zeit. f. exper. Path. u. Ther.; 1905 (2), 117). According to this theory the direction and speed of osmosis are determined by the difference in surface tension between the fluids on the two sides of a membrane, the fluid with the less surface tension passing towards the one with the higher tension. Surface tension differs from osmotic pressure especially in that the nature of the dissolved substance is of more importance than the quantity, *e. g.*, 1 gmw. of amyl alcohol lowers surface tension as much as 81 gmw. of methyl alcohol, although equivalent amounts of both produce the same effect on osmotic pressure. On this theory is built up a conception of physiological secretion and absorption, which considers that only fluids of lower tension than that of the blood enter it, *e. g.*, absorption from the gastrointestinal tract is favored because bile and peptone both lower surface tension, etc. For details see the original article cited.

² For further consideration of the subject of osmotic pressure in these relations see: Livingston, "The Rôle of Diffusion and Osmotic Pressure in Plants," University of Chicago Press, Chicago, 1903; Czapek, "Biochemie der Pflanzen," Jena, 1903. Also, Höber, Cohen, and Hamburger, all previously cited.

³ For full discussions of the nature of colloids see: Höber, "Physikalische Chemie der Zelle," Leipzig, 1902; Pauli, Ergebnisse der Physiologie, 1904 (III, Abt. 1), 155; Mann, "Physiological Histology," Oxford, 1902. The complete literature is collected and summarized by Aron in the Biochemisches Centralblatt, 1905 (3), pages 461 and 501. The relation of colloids to the problems of immunity is reviewed by Zangger, Cent. f. Bakt. (ref.), 1905 (36), 161.

of matter. Although at the present time the differences between the two classes do not seem so great, yet the same division is found useful in classification. By colloids Graham indicated those substances which were dissolved to the extent of showing no visible particles in suspension, but which either did not pass through diffusion membranes at all, or did so very slowly indeed, as compared to the crystalloid substances. Under certain conditions they tended to assume a sticky, glue-like nature, hence the name. (Many substances are now known which have the chief properties of the colloids and are therefore classified among them, but never are glue-like, *e. g.*, the colloidal metals, so that the name has lost some of its original significance.) The physical property which Graham particularly noted in the colloids, besides their non-diffusibility, was the tendency to assume various states of solidity. Not only can they be in solution, when he called them "sols" (when the solvent was water, "hydrosols"), but they can become quite firm although containing much water (then called "gels" or "hydrogels"). The gels may assume a firm, coagulated condition, the so-called "pectous" state, which state is permanent in that the gel form cannot be reobtained from the pectous modification. Finally the colloid can be in a dry, solid state, quite free from water, and then not a sol at all.

Included in the great class of colloids are all forms of proteids, and also gums, starch, dextrin, glycogen, tannin, chondrin, perhaps the soaps and lecithin, probably the enzymes, and also the greater number of organic dyes; also there are inorganic colloids, such as silicic acid, arsenic sulphide, hydrated oxide of iron, and many other similar compounds, besides the elements themselves, especially the noble metals which may exist in colloidal form. It will be seen at once that the chief constituents of the cells, in fact nearly all the primary constituents except the inorganic salts, are organic colloids, and therefore *the properties of the cells are largely dependent upon the properties of the colloids.*

In considering the characteristics of the colloids we at once meet the question—What distinguishes the colloids from the crystalloids, on the one side, and from suspensions or emulsions on the other? An enormous mass of literature has been piled up by the workers upon the problems here presented, and although the last word has not been said, yet a fair understanding of the conditions of solution has been reached, and many important properties have been discovered and explained. The sum and substance of our present conception of the nature of colloidal solution may be briefly summarized as follows :

It is possible for solid substances to be so divided among the particles of a solvent that they remain permanently in this condition, neither aggregating into masses nor separating out through the action of gravity. With some substances, as sugar, for example, the solid seems to divide up into its molecular form, each molecule being free from all others of its kind except during occasional contacts. Some other substances, as salt, go still further, and the molecule divides into two or more parts, which have different electric charges (*ionization*). The first of these classes of substances forms a solution which contains no particles visible by any known means, does not contain particles large enough to refract or reflect light impinging upon them, exerts a large osmotic pressure, but does not conduct electricity. The other, in which ionization has occurred, differs solely in its capacity to conduct electricity readily. Both are *true solutions* of crystalloids; the one which does not ionize is a *non-electrolyte*; the other, by virtue of its ionization, is an *electrolyte*, the ions carrying electric charges through the solution.

At the other end of the scale we have substances which are quite insoluble when in masses, but which, when very finely divided by mechanical means, can be suspended and uniformly distributed through a fluid without having any marked tendency to aggregate or settle out. Such *suspensions* or *emulsions* contain particles visible under the microscope, usually appear turbid, refract light, are non-diffusible, exert no osmotic pressure, and do not transmit electricity. Such mixtures are obviously very different from the true solutions above described.

Between these two extremes stand the colloids, which vary in their properties so that they approach sometimes the suspensions (*e. g.*, lecithin, or coagulated egg-albumin in colloidal suspension), and sometimes more nearly the true solutions (*e. g.*, dextrin). No sharp boundaries can be drawn between any of the members of the series. Indeed, one substance may present all the different stages under different conditions; to illustrate, arsenic sulphide may appear as a suspension in water, with such large aggregations of its particles that most or all of it can be removed by an ordinary filter. It may exist, however, in the form of a colloidal solution or suspension, which appears perfectly homogeneous to the naked eye, but when placed under the microscope, the fluid is found to be filled with minute particles in Brownian movement. Still other colloidal suspensions of the same substance may be obtained which with the best oil-immersion lenses show no particles of any kind, but when these solutions have a beam of light passed through

them it becomes visible because of the reflection of the light waves on the surfaces of solid particles that are suspended in the fluid, as a ray of sunlight becomes visible in a dusty room (Tyndall's phenomenon¹). Hence this solution, which even with the microscope appears as perfectly homogeneous as a salt solution, is in reality full of solid particles. Finally, still other solutions of arsenic sulphide may be obtained in which the particles are so fine as to diffuse like an ordinary solution of a crystalloid.

In a similar manner various other colloids may be found to show different characters, some agreeing with the properties of the typical suspensions, and some with the properties of the true solutions. They stand in an intermediary position, differing quantitatively in one way or another from the true solutions, but yet approaching them closely and sometimes almost indistinguishably resembling them. For the most part, however, the colloids show characteristics decided enough to entitle them to separate classification, and to make any confusion with the crystalloids impossible.

The Characteristics of Colloids.—The chief properties of the colloids are, then, as follows :

Amorphous Form.—This, like almost all other "colloidal properties," is not absolute, for in egg-albumin, hemoglobin, and various globulins we have proteids which in every respect are typical colloids, yet they form crystals readily and abundantly. Oxyhemoglobin, the molecular weight of which is calculated at about 14,000, exhibits Tyndall's phenomenon, and will not pass through a very fine porcelain filter, and therefore resembles the colloids decidedly, yet it forms beautiful crystals. The very fact that crystals are formed, Spiro points out, is proof that when in solution the individual molecules must have been free and separate, for otherwise they could scarcely unite in the definite spatial relations necessary to produce crystalline forms.²

¹ The so-called ultra-microscopic method of studying minute particles, devised by Siedentopf and Zsigmondy, depends upon the same phenomenon. In this method the particles are illuminated in the microscopic field by an intense ray of light, and the reflection of light causes the particles to appear as minute luminous points. Particles as small as 0.005μ can be detected in this way, and ordinary colloidal solutions of albumin appear filled with moving particles.

² This indicates that in colloidal solutions the molecules may be free, and not necessarily aggregates. This is perhaps only true for the substances of very great molecular dimensions, such as the proteids; the colloidal solutions of substances with smaller molecules having the molecules united in large groups. On this basis the essential difference between colloidal and true solutions is merely one of the size of the free particles.

Graham's term of "crystalloid," therefore, does not strictly express the distinction he intended, or, rather, the distinction he intended does not exist in so decided a way as he imagined. With these few exceptions, however, the colloids do not present any typical structure, and are not crystalline under any visible condition. But when they are made insoluble by chemical means they may, under certain conditions, produce rather characteristic non-crystalline structures, a matter that will be discussed in a subsequent paragraph.

Solubility.—Although we speak of "colloidal solutions," this term does not commit us to the theory of the identity of the solution of colloids with that of crystalloids. We have above stated what seems to be a fair view of the matter as shown by many methods of experimentation. Most colloids seem to be, in fact, suspensions of masses of molecules, or perhaps of very large single molecules, and a true solution is likewise a suspension of single molecules or of ions. When the aggregations of molecules are sufficiently large, we have an ordinary suspension; but a single proteid molecule is as large as a very great number of molecules of such substances as sugar (crystalloid); or tannin, $C_{14}H_{10}O_9$ (colloid); or calcium carbonate (insoluble, suspension); and it would be strange if a true solution of a proteid did not behave in many particulars like a suspension of molecular aggregates of dimensions similar to the dimensions of proteid molecules. Nearly all colloidal solutions show Tyndall's phenomenon, which demonstrates the existence of particles in suspension large enough to reflect light from their surfaces. Most of the colloids are held back by very fine filters to a greater or less degree; some are almost entirely retained by a hardened paper filter, while others pass through the finest-pored clay filters. Furthermore, the metallic colloids, such as those of platinum, gold, and silver, are unquestionably suspensions of finely divided particles of metal, yet they exhibit all the typical phenomena of colloids, passing through many sorts of filters, and even accomplishing the same hydrolytic changes as many enzymes.

It must also be mentioned that the solvent is probably an important factor in determining the colloidal or non-colloidal nature of a substance; *e. g.*, soaps form true solutions in alcohol and colloidal solutions in water; gelatin forms colloidal solutions in water but not in ether, whereas rubber forms colloidal solutions in ether but not in water.

Closely related to solubility is the phenomenon of *imbibition* (the "Quellung" of German writers), which may be defined as

the taking up of a fluid by a solid body without chemical change. Not all colloids possess this property, but it is shown by most of the organic colloids, particularly the proteids. Fick distinguishes capillary, osmotic, and molecular imbibition, the latter of which is the form exhibited by colloids, and it occurs independent of the existence of pores or other preformed spaces in the imbibing body. The imbibition of water by colloids is more than a simple mechanical process, for it is accompanied by a contraction in the total volume of solid and water, and by the evolution of heat. On the other hand, the physical properties of an aqueous colloidal solution show that the colloid is not chemically combined in the form of a hydrate. To describe this peculiar relation Hofmeister and Oswald recommend the term "mechanical affinity." Hardy has shown that water held in a gelatin jelly cannot be removed by great pressures (400 pounds to the square inch), but after the nature of the jelly is so changed by formalin that it is no more liquefiable by heat, the water can be easily expressed from the loose meshwork that is formed. It would seem from this that the imbibition and retention of water by colloids may be closely related to surface phenomena. Hofmeister has shown that organized animal tissues obey the same laws of imbibition as do simple gelatin plates, and probably this phenomenon of colloids is very important in physiological processes.

Non-diffusibility.—The lack of power to pass through animal and parchment membranes, which was Graham's starting-point in the study of colloids, is also only a relative condition. This is shown by the following figures giving the relative time required by the same amount of different substances to pass through a certain diffusion membrane :

Sodium chloride	2.33
Sugar	7.00
Magnesium sulphate	7.00
Proteid	49.00
Caramel	98.00

This difference of time is so great, however, as to permit of separation of salts from proteids, etc., by dialyzation, a process in constant use. Primarily the ability to diffuse through a given membrane requires that the diffusing substance be soluble in the membrane. Diffusion membranes are always composed of colloids, *e. g.*, animal bladders, or parchment, which is a colloidal cellulose. Crystalloids are generally soluble in colloids, while colloids are little or not at all soluble in other colloids, and hence do not diffuse through one another and therefore they

cannot permeate diffusion membranes. For example, if a stick of agar jelly be placed in a solution of ammoniated copper sulphate (a crystalloid), and another be placed in a solution of Prussian blue (a colloid), it will be found that the copper solution penetrates the agar rapidly, while the colloidal solution of Prussian blue does not penetrate it at all. This property is of great importance, undoubtedly, in keeping different colloidal constituents of the cell in given localities within its protoplasm, *e. g.*, the oxidizing ferments seem to be chiefly localized within the nucleus; the colloidal glycogen remains where it is formed in the cytoplasm, unable to escape from the cell, whereas the crystalloidal sugar from which it is formed and into which it is converted, diffuses rapidly into or out of the cell.

The **osmotic pressure** of the colloids is so small that some investigators doubt that colloids really do exert any osmotic pressure by themselves. They would explain such small positive results as have been obtained by assuming the presence of contaminating substances, a criticism that is well grounded on the fact of the extreme difficulty of obtaining colloids in a pure state. The closely related phenomena of *diffusion*, *depression of freezing-point*, and *elevation of boiling-point*, are also exhibited by colloids to but an extremely slight degree. For example, in one experiment, the dissolving of from 14 per cent. to 44 per cent. of egg-albumin in water lowered the freezing-point but 0.02° to 0.06° ; and some other colloids have even less effect. But the results of the latest and best experiments seem to indicate that the trifling effects of colloids upon osmotic pressure and upon freezing- and boiling-points observed in colloidal solutions are due to the colloids themselves, although it may possibly be that some of these effects are due to the high surface tension and cohesion affinity of the colloids. In all cellular processes accompanied by manifestations of osmotic pressure or diffusion, however, the crystalloids may be considered as almost entirely responsible.

Electrical Phenomena.—As colloids do not separate freely into ions when dissolved, they do not conduct electricity appreciably. However, when an electric current is passed through water containing colloids in solution, the colloidal particles tend to pass to one pole or the other. Most colloids move toward the anode. This phenomenon, *cataphoresis*, is also generally exhibited by suspensions, and hence in this particular the colloids resemble suspensions rather than solutions. Helmholtz has explained the movement of the suspended particles as due to the accumulation of electrical charges upon the surfaces of two

heterogeneous media when in contact. The nature of the charge depends upon both the suspended substance and the fluid; *e. g.*, sulphur or graphite particles suspended in water assume a negative charge and move toward the anode, but when suspended in oil of turpentine they become positively charged and move toward the cathode. Water has such a high *dielectric constant* that most substances in water become negatively charged as compared with the water, and move toward the positive pole or anode.

Hardy has observed that colloidal solutions of coagulated proteids move toward the anode when in alkaline solution, and toward the cathode when in acid solution. This peculiar property of proteids suggests that perhaps simple surface phenomena do not suffice to account for the electrification of all colloid particles. Knowing the peculiar amphoteric character of proteids, which is probably due to the presence of both NH_2 and COOH groups in the molecule, we can readily believe that in an acid solution the NH_2 radicles are combined with the acid, leaving the COOH radicles free. The molecule would then have acid properties, and could dissociate into an acid H ion and a basic or electrically positive colloid ion. The colloid ion would then go toward the negative pole slowly, because of its great size. Were this true, however, we might expect the colloidal solutions to show more conductivity than they do, but possibly on account of the very large size of the proteid molecule too few H ions are liberated to produce much effect, and also, ionization may be much less in a neutral solution than in an acid or alkaline one. Electrification of suspensions of platinum, gold, or powdered glass could hardly be explained on this basis, unless we assume that the water or other solvent united with the particles becomes ionized. Quite possibly we have both ionization and cataphoresis occurring, the former in the case of some compounds, the last in the case of elements or perfectly insoluble substances held in suspension.

Surface tension, which may be described as *the force with which a fluid is striving to reduce its free surface to a minimum*, is highly exhibited by colloids as compared with crystalloids. The phenomenon of cataphoresis depends upon the existence of a high surface tension, and it is this same property that explains the ability of colloidal particles to stay suspended in a fluid which has a much lower specific gravity than the solid. The formation of emulsions and the spreading out of oil upon the surface of water also depend upon surface tension. Ameboid movement may be attributed to changes in surface tension, as also

may phagocytosis. (The relation of surface tension to these processes will be considered under the subject of Inflammation.)

The effect of colloids upon chemical processes going on within their solutions or gels is surprisingly small. Salts in solution in a thick gel of agar or gelatin will diffuse almost as rapidly as in water;¹ they will also ionize as rapidly as in watery solutions, and chemical reactions occur with the same speed and completeness as if the colloids were absent. Furthermore it makes little difference whether these processes are measured in a colloid solution that is liquid, or after it has set in the gel form. These facts merely indicate that the colloids do not greatly impede the movements of molecules or ions in solutions. On the other hand, as before mentioned, colloids diffuse little or not at all into each other. Hence, in the cell the colloids are quite fixed in their positions, whereas the crystalloids may wander about freely, and this arrangement is certainly of great importance in biologic processes. Pauli suggests the probability that the fixation of the colloid causes the cell to have different properties in different parts, and so various reactions may occur independently in different areas of the cytoplasm. The possibility of the correctness of this view is increased when we consider that the enzymes are colloids, for there is much evidence to show that they are distributed in just such an uneven manner within the cells.

Although colloids permit the passage of dissolved crystalloids through them, they greatly interfere with the movement of larger particles. This property accounts for the ability of colloids to hold many insoluble substances in such extremely fine suspensions that they seem superficially to be in true solution. If, for example, phosphoric acid is added to a solution of casein in lime-water, the calcium phosphate formed does not precipitate. It is not in solution, however, but rather exists as a suspension of very finely divided particles of the salt which the colloid keeps from aggregating into particles large enough to be visible or to overcome the viscosity of the fluid and sink to the bottom. Probably in this way many substances, including calcium salts, are carried in the blood, held in permanent suspension by the proteids. Substances thus finely divided will have extremely large surface area for reactions, and, therefore, will undoubtedly undergo changes with considerable rapidity and facility, although not in solution.

¹The retarding influence of colloids upon diffusion has, however, been generally underestimated, according to the most recent researches. (See Meyer, Hofmeister's Beitr., 1905 (8), 393; Nell, Ann. d. Phys., 1905 (18), 323; Flexner and Noguchi, Amer. Med., 1906 (1), 154.)

Precipitation and Coagulation of Colloids.—Because of the rather slender margin by which the colloids are separated from the suspensions, their persistence in solution is generally in a rather precarious condition. Relatively slight changes suffice to throw the colloids out of solution, and when once precipitated, they are often incapable of again dissolving in the same solvent. Solutions of albumin may undergo spontaneous coagulation on standing for some time, and agitation rapidly produces the same effect in many proteid solutions. Some inorganic colloids are as readily coagulated as the proteids. A comparatively small rise in temperature, less than to 50°C. with some proteids, renders the proteid perfectly insoluble. Furthermore, we have coagulation of proteid solutions by enzyme action. The inorganic “colloidal suspensions” may be precipitated by the addition of very small quantities of electrolytes. Colloidal solutions of the type of the proteids are not so readily precipitated by most electrolytes, but if to the solution large quantities of crystalloids are added, the proteid molecules are practically crowded out of solution, as in the “salting-out” process used in separating proteids by ammonium sulphate and other salts. The effect of heat upon different colloids is peculiar, in that some varieties, as silicic acid, aluminium hydrate, and many proteids are rendered so insoluble that they cannot again be dissolved in any fluid without first being modified in some way; whereas colloids of the type of gelatin and agar are made more soluble by heat. The change of colloids into insoluble forms, the “*pectous*” condition of Graham, requires the presence of water, for the dry colloids may be heated to relatively high temperatures without losing their solubility. On the other hand, dehydration of colloids while in solution will result in their precipitation and coagulation, as occurs in proteid solutions when alcohol is added.

Colloids are precipitated by many electrolytes, apparently through the formation of true ion compounds, one or both of the ions of the electrolytes uniting with the colloid ion; although some writers, as Spiro, believe that the combination is merely an additive one between entire molecules. Mathews¹ has advanced the theory that the *solution tension* of the salts is the chief factor in determining the precipitation of colloids by electrolytes. Colloidal particles have a high surface tension which is always tending to reduce the volume of the particle, and in colloidal solutions this is constantly opposed by the force of solution tension. If the solution tension of the salt is of such

¹ American Journal of Physiology, 1905 (14), 203.

a charge as to increase the solution tension of the colloid, the solubility of the colloid is increased, but if it is of opposite charge to that of the colloid, the surface tension is no longer counterbalanced by the solution tension, and the bulk of the molecule is reduced, while its weight remains the same; hence it falls out of the solution. A similar effect may be produced by the union of several molecules by a polyvalent ion—their total surfaces will then be much reduced as compared with what it was when they were separate, and so the surface energy is no longer sufficient to keep them in solution. In general, precipitation of colloids results from the reduction of the surface in proportion to the mass, because of an aggregation of the particles; this may be brought about by changing the surface electrical conditions, by uniting the molecules chemically, or by reducing the amount of the solvent.

The Structure of Colloids and of Protoplasm.—Two very different sorts of substances are usually included under the term colloid, because they show the essential features of colloids in most respects; but as in many other respects they are quite unlike each other, it may be well to distinguish between them in some way. As a type of one class we may take gelatin; of the other, such a substance as colloidal arsenic sulphide. Gelatin solutions form gels upon cooling or evaporation, and redissolve when heated or when more solvent is added. Arsenic sulphide does not form gels upon cooling, and when solidified in any way, does not redissolve. In addition, the gelatin type is very viscous, and is not coagulated by the presence of salts unless these are added in large amounts; while the other type does not render the fluid in which it is dissolved appreciably more viscid, and it forms a precipitate immediately if minute amounts of electrolytes are introduced. As the former type resembles in many details the true solutions, while the latter approaches more closely to the suspensions, it has been proposed to distinguish them by the terms "colloidal solution" and "colloidal suspension."¹ Of the two types, the colloidal solutions are by far the more important in biological considerations, since the colloidal suspensions are usually prepared artificially and seldom occur in nature, *e. g.*, Bredig's colloidal suspensions of the noble metals.

The colloidal solutions of proteids, which constitute the chief part of every cell, are of two types—one, such as albumin, forms a coagulum when heated, which, under ordinary conditions is not reversible; that is, it does not again go into

¹ Noyes, American Chemical Journal, 1905 (27), 85.

solution. Gelatin, however, becomes more fluid when heated, and when cooled, it forms a gel which is readily reversible to the soluble form under the influence of heat. Agar is another familiar example of this heat-reversible type. Within the cell, so far as we know, occur only the first type, the proteids that form non-reversible coagula.

An extensive study of the physical structure of the colloids has been made by Hardy.¹ As long as the colloid is in solution it is structureless, although, as before mentioned, the existence of free solid particles can be demonstrated by certain optical methods. The solution is homogeneous, and although perhaps viscid, still it is a typical solution. Such solutions can become solid, either by the effect of temperature, of certain chemical fixing agents, or physical means. It was found by Hardy that in undergoing this solidification there occurred a separation of the solid from the liquid, the solid particles adhering to form a framework holding the liquid within its interstices. Heat-reversible gels show no structure until they are made irreversible by hardening agents, etc.; *e. g.*, a jelly of gelatin appears structureless, but when treated with formalin or other fixing agent, the structural appearances described below appear. The figures formed by the framework vary according to the nature and concentration of the colloid and of the solvent, and also upon the fixing agent used, the temperature, and the presence or absence of extraneous substances. In general, however, the figures obtained in the solidification of proteid solutions by fixing agents, such as bichloride of mercury or formalin, *bear a striking resemblance to the finer structures of protoplasm* as described by cytologists. There is produced an open network structure with spherical masses at the nodal points, or minute vesicles hollowed out in a solid mass, or a honeycomb appearance, or, when the concentration of the colloid is very slight, perhaps there is only a precipitation of fine granules of proteid such as we often see in histological preparations of edematous cells and tissues. All these forms seem to depend chiefly upon the concentration of the colloid. The important fact is that when the chemicals ordinarily used as fixatives of cells for histological purposes act upon solutions of colloids that are perfectly homogeneous, they produce very constant and characteristic formations which recall at once the structures found in the protoplasm of hardened cells. Moreover, the use of different fixing agents, such as osmic acid, formalin, and bichloride of mercury, produce just the same differences in the

¹ Journal of Physiology, 1899 (24), 158.

structure of colloidal solutions that they produce in the protoplasm of cells hardened by them. Neither are the appearances seen in unfixed specimens reliable indications of the true structure of the living protoplasm. Granules of secretion may disappear after or during the death of the cell (*e. g.*, glycogen) or they may swell up (*e. g.*, mucin granules) thus giving the appearance of a network or honeycomb which is then incorrectly ascribed to the protoplasm itself. Death of the cells, even when not produced by external influences, seems to be accompanied by coagulation of some parts of the cell constituents, and hence a cell examined in anything but its normal living condition, an extremely difficult matter, will not present a true idea of how it appears and is composed while in that condition.

If, with these facts in mind, we consider the theories of morphologists as to the finer structure of the cell protoplasm based upon studies of cells fixed in various hardening agents, it becomes evident that the possibility that the "foam structure" advocated by Bütschli, or the "thread," "reticular," and "pseudo-alveolar" structures of Fromann, Arnold, Reinke, and others, are all simply the effect of fixatives upon colloid solutions seems very real. The objection always advanced to these theories of protoplasmic structure, namely, that the structures described were artificial productions, not present in the normal living cell, and variously described and interpreted by different investigators because each worked with a different hardening fluid or different technic, is strongly supported by these observations upon colloids. The possibility that the living protoplasm is homogeneous still remains open. This matter will receive further consideration in the next section.

THE STRUCTURE OF THE CELL IN RELATION TO ITS CHEMISTRY AND PHYSICS

It is obviously impossible to separate nuclei, nucleoli, cytoplasm, and cell membranes from each other and to isolate them in quantities sufficient for analysis, and therefore we are still quite uncertain as to just the chemical differences that exist between them. That there are differences is certain, and by means of micro-chemical reactions, by comparing analyses of cells in which nucleus or cytoplasm predominate, and by studying their physico-chemical relations to one another, we have arrived at more or less tangible ideas on the question of the relation of the structural elements of the cell to its composition.

THE NUCLEUS

Although the nucleus presents morphologically a sharp isolation from the cytoplasm, and displays equally sharp tinctorial differences, it is probable that chemically the differences between nucleus and cytoplasm are quantitative rather than qualitative. The characteristic affinity of certain elements of the nucleus for basic stains depends upon the presence in the nucleus of nucleoproteids in large proportion, and to a limited degree nucleoproteids are characteristic of nuclei. Their affinity for basic dyes depends upon their acidity, which is due to the nucleic acid radicle. *In inverse proportion to the degree to which this acidity is neutralized by proteid groups in the nucleo-proteid molecule, the nucleo-proteid will show affinity for basic dyes.* For example, the heads of spermatozoa contain nucleic acid bound to little or no proteid, hence they are very acid, have a corresponding affinity for basic dyes, and appear intensely stained by hematoxylin, etc. Ordinary chromatin threads of nuclei appear to contain somewhat more proteid in their nucleoproteid molecules, and hence stain less intensely than do the spermatozoa heads, except when in karyokinesis, when the chromatin nucleoproteid seems to approach that of the spermatozoa in acidity. We also have nucleoproteids with the nucleic acid so thoroughly saturated by proteid that they do not stain at all by basic dyes, and these seem to exist principally in the cytoplasm, and also to form the ground-substance of the nuclei, occupying the spaces between the chromatin particles (this achromatic substance of the nuclei is called *linin* or *plastin* by some cytologists). Besides the chromatin and the nucleoli, there is a peculiar chromatophile substance, suspended in the finer part of the nuclear structure in the same manner as the chromatin itself is in the coarser portions; this was called *lanthanin* by Heidenhain,² and is probably similar to the substances also described as *parachromatin* and *paralinin*. Undoubtedly the other forms of proteids found in the cell, such as globulin, albumin, and nucleoalbumin, exist both in the nucleoplasm and in the cytoplasm, the essential difference being that the proportion of nucleoproteid is much greater in the nucleus, and that the nucleoproteids of the cytoplasm contain relatively more proteid in proportion to the nucleic acid than do the nucleoproteids of the nucleus. As nucleoproteids are little affected by peptic digestion, it is possible

¹ Earlier literature by Albrecht, "Pathologie der Zelle," Lubarsch-Ostertag Ergeb. der allg. Pathol., 1899 (6), 900.

² Festschr. f. Kölliker, 1892, p. 128.

to isolate nuclear elements, especially the chromatin, for analytic purposes, and it has been demonstrated by this means also that nuclein is the chief constituent of the staining elements. The distribution in the nucleus, of the other primary constituents of the cytoplasm, such as lecithin, cholesterin, and inorganic salts has not yet been worked out, except that Macallum¹ has found that nuclei contain no chloride, as indicated by their not staining with silver nitrate, and also no potassium.²

Nucleoli, which not all varieties of nuclei possess, differ from the other nuclear structures in having an affinity for acid rather than for basic dyes.³ Their chemical composition has not been ascertained. Zacharias considers the nucleoli as composed of nuclein well saturated with proteid, because of its staining reactions and its relative insolubility in alkalies, and classes it with plastin or linin, which forms the achromatic part of the nucleus and is also present in the cytoplasm. Macallum⁴ found that they reacted for organic phosphorus microchemically, but less strongly than did chromatin fibers.

The **nuclear membrane** is an uncertain structure, at times dense and staining as if formed of a layer of chromatin, in other cells staining like the cytoplasm with which it seems to be continuous, in most cells disappearing during karyokinesis, and in some protozoa being entirely absent. Naturally the composition of the nuclear membrane is unknown, but it is probable that it acts as a diffusion membrane of partially semipermeable character, maintaining different conditions in nucleus and cytoplasm.

Functionally the nucleus is the essential element of the cell; an isolated nucleus may be able to develop new cytoplasm, but isolated cytoplasm soon disintegrates, although it may manifest life for some time by movement and chemical activities. A popular theory is that synthetic, constructive processes occur in the nucleus or under the influence of its products, but to what the nucleus owes these hypothetical powers is unexplained. More tangible are the theories based upon the work of Spitzer, Loeb,⁵ Lillie⁶ and others which show that the oxidative processes of the cell depend upon the nucleus, hence portions of the cell cut away from the nucleus undergo asphyxiation. As

¹ Proceedings of the Royal Society, 1905 (76), 217.

² Jour. of Physiol., 1905 (32), 95. The reliability of the method used by Macallum has been questioned by Tracy (Jour. Med. Research, 1906 (14), 447).

³ Nucleoli of nerve-cells are an exception, being basophilic.

⁴ Proc. of the Royal Society, 1898 (63), 467.

⁵ "Studies in General Physiology," Chicago, 1905.

⁶ American Journal of Physiology, 1902 (7), 412.

Loeb says, "By cellular structure we understand the fact that there must be a definite maximal distance between the elements of the protoplasm and the nearest nucleus."

It should be mentioned that certain cells, such as bacteria and algæ, seem to have no true nuclei, but Macallum¹ found that the forms he examined gave reactions for phosphorus and iron in a similar way to the nucleoproteids of a nucleus, suggesting that in such cells the nuclear elements are diffused through the cell rather than differentiated. To quote Wilson: "The terms 'nucleus' and 'cell body' should probably be regarded as only topographical expressions, denoting two differentiated areas in a common structural basis."

Because of the relative acidity of the nuclei they are electrically negative to the cytoplasm, particularly when in karyokinesis. Sperm-heads in isotonic cane-sugar solution move rapidly—2000 microns a minute—toward the anode, when a current is passed through the solution; and leucocytes also go toward the anode under the same conditions, the rate depending upon the proportion of nucleoplasm and cytoplasm, large leucocytes sometimes even going slowly toward the cathode. The Sertoli cells of the testicle, which have a round mass of cytoplasm with a number of miniature spermatozoa heads at one side, orient themselves in the current so that the side or end containing the spermatozoa drags the mass of cytoplasm toward the positive pole.

THE CYTOPLASM

The cytoplasm, as before mentioned, contains all of the primary cellular constituents, and also such secondary constituents as the particular cell possesses. Nucleoproteids are undoubtedly present in unknown proportions, but with the nucleic acid well saturated by proteids, and perhaps also to a large extent combined with carbohydrates to form the glyconucleoproteids. Sometimes the nucleoproteids of the cytoplasm may be partly of the unsaturated class, and show an affinity for basic stains, as in the case of the Nissl bodies of the nerve-cells, and perhaps also the cytoplasm of plasma cells. The great question concerning the cytoplasm is its structure—whether homogeneous, alveolar, areolar, fibrillar, foam-like, or granular. On a previous page have been mentioned the experiments of Hardy, which show that homogeneous solutions of proteid, when fixed by the same reagents as are used in the customary fixation of histological materials, may show quite the

¹"Studies from the University of Toronto," 1900.

same microscopical structures as are shown by the cytoplasm of cells. Network, foam, and alveolar structures are produced in albumin and gelatin solutions when they are hardened by bichloride of mercury, osmic acid, formalin, etc., and the same characteristic differences that are produced in cells by these different reagents are likewise produced in the hardened proteid solution. Proteid structures hardened under strain form radiating structures resembling centrosomes and the radiating threads seen in cells. If elder pith is saturated with proteid solutions and then hardened, sectioned, and stained by the usual methods, appearances resembling closely the structure of a hardened cell may be found in the spaces of the pith—even a central, nucleus-like mass may be suspended in a network of anastomosing threads. These and many other experiments indicate that much of the work done on cell structure by means of studies of hardened cells cannot be considered of value in deciding the structure of living cells; but, nevertheless, the fact remains that many cells that can be observed while alive and uninjured under the microscope are seen to have a definite structure in the cytoplasm, *e. g.*, sea-urchin eggs, which show a characteristic alveolar structure.

A compromise view of the structure of protoplasm (and cytoplasm in particular) which takes account of what appear to be facts brought out on both sides of the question, is that while in some cells definite structural arrangements of the cytoplasm exist, in most cells, and to a large extent even in cells showing a cytoplasmic structure, the proteids are in a homogeneous solution; most of the structures seen in fixed cells, except the chromatin threads, nuclear membrane, nucleoli, and centrosomes, are produced by the coagulation of the proteids, and are not present during life. When a framework does exist, it is a fair inference, by analogy with the cell membrane and the stroma of the red corpuscles, that the cell lipoids are largely responsible for its formation, and that they form a prominent part of its composition. This question of the presence or absence of structure in the cytoplasm is of more interest than as a mere morphological problem, for if the cytoplasm is subdivided into innumerable little chambers, each surrounded by a membrane, it is probable that processes of diffusion and conditions of osmotic pressure will be very different from what they would be if the cytoplasm were a simple homogeneous colloid solution, like a lump of semisolid gelatin or agar. In such colloidal masses diffusion and osmosis go on almost as if there were no colloids in the solvent at all, whereas most membrane structures that

are found in living tissues seem to have a decidedly semipermeable character.

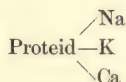
From what we know at the present time of intracellular physics and chemistry there is no necessity for assuming that semipermeable septa exist within the cell. All the intracellular processes with which we are familiar *could* go on without such structures. It is not necessary to assume a compartment structure to explain the possibility of different chemical reactions going on in different parts of the cell at the same time, for most of the cell reactions seem to depend on enzymes, which we know are not readily diffusible in solutions of colloids, and, therefore, might remain fixed without requiring any enclosing walls or retaining framework. Certainly, many cells are free from structural cytoplasm, for we see particles of solid matter moving about within them quite freely. In some cells the nuclei migrate about in the cell, as also do digestive and excretory vacuoles, which motion would seem to be rather destructive if the protoplasm had a structure at all permanent.

When a portion of the cytoplasm is cut free from the body of certain cells it at once forms a round drop, just as any insoluble fluid would do in another of different surface tension, and not at all as if it were bound into a fixed structure by a framework. Other cells, however, retain their form under the same conditions. The structure of even so evidently complicated a cytoplasm as that of striated muscle-fibers is in doubt; a classical observation on this point is the passage of a minute worm through the substance of a muscle-cell, its progress being as unimpeded as if there were no such things as disks, bands, rods, and striæ in the cell. Many features of ameboid movement also seem to indicate that the cytoplasm follows much the same laws as a drop of fluid in a heterogeneous medium, for we can make a drop of mercury or of chloroform in water, or of oil in weak alcohol, react to various stimuli in much the same way that an ameba would.

The question of structure in the nucleus is quite a different matter, in so far as the chromatin threads and the nucleolus are concerned. In ameboid movement the nucleus seems to play a passive rôle and to be dragged about by the cytoplasm, indicating quite a high degree of rigidity. It is probable, however, that the achromatic portion between the chromatin threads and granules has much the same structure or lack of structure as the cytoplasm.

The *inorganic salts* seem to be, at least in part, contained in the cells in chemical combination rather than in simple solution in the water of the cell. There is much evidence indicating

that they form with the proteids ion compounds, which may be altered under various conditions. For example, Loeb found that muscles placed in solutions of potassium salts took up much water, whereas if placed in a solution of calcium salts they lost water, exactly as soaps do when potassium or calcium ions are substituted for the sodium ions in a sodium soap. He has suggested that we have in the cells a proteid-ion compound, after this order,



and that if, in the surrounding fluid, a great excess of one of these ions is present, it may displace the others by mass action, forming a proteid with all or most of the ions of one kind, and, therefore, decidedly abnormal. Many features of cell physiology seem explainable on these grounds, and the reader is referred to Loeb's collected works for further discussion.¹ In any event it is important for the cell that the proportion of the inorganic constituents be maintained in rather constant conditions of quality and quantity.²

The various secretory granules, fat-droplets, pigment-granules, glycogen granules, keratin, etc., that may lie in the cytoplasm, are inconstant constituents, varying with different cells, and under varying conditions in the same cells, and lie beyond the scope of our discussion of the general composition of the cell.

THE CELL-WALL

The cell membrane in most animal cells is inconspicuous structurally, but in discussing osmosis it was shown that it is of the greatest biological importance. There is no direct chemical or microscopical evidence at hand showing the composition of the animal cell membrane, but by observations on its behavior when the cells are in solutions of different sorts, facts have been collected indicating that lecithin and cholesterin, and probably the allied fat-like bodies, "protagon" and cerebrin, are prominent constituents. The substances that diffuse through most cell walls are just the substances that are soluble in or dissolve these lipoids, *e. g.*, alcohol, chloroform, ether, etc., and it is

¹ "Studies in General Physiology," 1905.

² According to Macallum, potassium can be demonstrated by microchemical methods in the cytoplasm and extracellular structures, but this could not be confirmed by Tracy (Jour. Med. Research, 1906 (14), 447), who questions the reliability of the method, and states that, if the reaction indicates anything, the potassium is chiefly in the nucleus.

probable that the anesthetic effects of many of these substances depend in some way on their fat-dissolving power and the large proportion of lipoids in nerve-cells. These observations were made first by Overton¹ and Meyer.² Of particular interest for our purpose are Overton's observations on the effects of dyes on living cells. The best known vital stains (*i. e.*, stains that will enter the living cell without requiring or causing injury to it) are neutral red, methylene blue, toluidin blue, thionin, and safranin. If uninjured cells, *e. g.*, frog eggs, are placed in watery solutions of these dyes they soon become filled with the coloring-matter, which seems to penetrate the cell membrane quite uniformly at all points; if the dyed eggs are then placed in clear water, the stain diffuses out again, showing it to be simply absorbed, rather than chemically combined. In contrast to these stains the sulphonic acid dyes, such as indigo carmine and water-soluble indulin, nigrosin, and anilin blue, do not penetrate the living cell at all. Overton tested the solubility of these last-named dyes and found them all insoluble in oils, fats, and fatty acids; but the first group, those staining living cells, were readily soluble in lecithin, cholesterolin, "protagon," and cerebrin, the so-called cell lipoids. Furthermore, if crumbs of lecithin, "protagon," or cerebrin were placed in very dilute watery solutions of these dyes, they were found to absorb from the water the vital stains, but not the others, which indicates that stains that penetrate living cells are more soluble in lecithin than they are in water.

Many facts indicate that the delicate membrane of animal cells has the features of a semipermeable membrane, to the extent of permitting certain substances to diffuse through and not others. Had it the property of many of the artificial semipermeable membranes, of letting water pass through but holding back almost absolutely all crystalloids, the result would be the development of an enormous disproportion in the pressure between the inside and the outside of the cell. Furthermore, the exchange of nutritive material and excretion products between the blood and the cells would be impossible. But permitting some substances to pass through the cell membrane results in their accumulation within the cell, until they are in sufficient concentration to neutralize the osmotic pressure exerted on the outside of the cell. As evidence of this elective permeability we have the fact that the proportion of certain salts within the cell is quite different from what it is in the

¹ Jahrb. f. wissenschaftl. Botanik, 1900 (34), 669.

² Arch. f. exp. Path. u. Pharm., 1899 (42), 109.

fluids bathing them; *e. g.*, animal cells generally contain more potassium and less sodium than the fluids surrounding them. The inorganic constituents of red cells are totally different from those of the plasma, the corpuscles not containing any calcium at all, while the magnesium seems to enter them freely; in other words, the red corpuscle seems to be impermeable to calcium and permeable to magnesium. If the salts in a corpuscle are in smaller proportion than in the surrounding fluid, it indicates that the cell membrane is not freely permeable for them; if in greater proportion, that some constituent of the cell is holding them in combination, possibly as ion-proteid compounds. Probably inorganic salts are present in the cell by virtue of both physical and chemical influences, some simply diffusing in and out, others combining with the proteids and being held chemically.

The **intercellular substance** varies greatly in different tissues. In the case of the supportive tissues it is the important element, and the cells seem to exist chiefly for the purpose of forming and keeping it in repair as it is worn out. In the epithelial and secreting tissues, however, the intercellular substance is reduced to a minimum, except in so far as a cement substance is required, and the cells generally lie in almost immediate apposition. It is probable that there is a greater or less amount of cement substance, even between the most closely applied cells, and this substance seems to be related to mucin. It can generally be brought out by staining with silver nitrate, and Macallum¹ points out that this reaction is merely a micro-chemical test for chlorides, and indicates that the cement substance contains them in larger proportion than does the cytoplasm.

¹ Proceedings of the Royal Society, 1905 (76), 217.

CHAPTER II

ENZYMES

EVERY cell is constantly accomplishing an enormous number of chemical reactions of varied natures, at one and the same time ; how many we do not know, but the score or more that we do know to be constantly going on in the liver cell, for example, are probably but a part of the whole. Furthermore, reactions take place between substances that show no inclination to affect each other outside the body, and proceed in directions that we find it difficult to make them take in the laboratory. Sugar is being constantly oxidized into carbon dioxide and water, a decomposition that requires high degrees of heat or powerful chemicals to bring about in the reagent glass. Proteids are being continually broken down into urea, carbon dioxide, and water ; yet to split proteids even as far as the amino-acid stage requires prolonged action of concentrated acids or alkalies, or superheated steam under great pressure.¹ But all the time in the cell a multitude of equally difficult changes is going on at once, within its tiny mass, always keeping the resulting heat within a fraction of a degree of constant, and the resulting products within narrow limits of concentration. We have already indicated the means used to keep the concentration of the cell products within safe limits ; namely, the processes of diffusion and osmosis and their modification by the cell structure. The forces that bring about the chemical reactions reside, we say, in enzymes, although in so doing we only shift the attribute formerly conceded to the cell, to certain constituents of the cell whose nature and manner of action are equally unknown. When the only enzymes that were known were limited to those secreted from the cell, and found free in fluids, such as pepsin and trypsin, the chemical changes that went on in the cell were ascribed to its "vital activity." Buchner, by devising a method to crush yeast cells, and finding the expressed cell contents able to produce the same changes in carbohydrates that the cells themselves did, proved the existence within living cells of enzymes similar to those excreted by certain cells, and

¹ For a fuller consideration of these phases of cell activity read Hofmeister, "Die chemische Organisation der Zelle," Braunschweig, 1901.

substantiated the belief of their existence that had become general before it was thus finally corroborated. Growing out from this and subsequent experiments has come a larger and larger amount of evidence that many of the chemical activities of the cells are due to the enzymes they contain, until now the point is reached where one may rightfully ask if cell life is not entirely a matter of enzyme activity. There are certain facts, however, which seem to indicate that there are some essential differences between cells and enzymes. One of the most important of these is the difference in the susceptibility to poisons of enzymes and cells. Strengths of antiseptics that will either destroy or inhibit the action of living cells, such as alcohol, ether, salicylic acid, thymol, chloroform, toluol, and sodium flouride, will harm free enzymes in solution little or not at all. This fact has been of great assistance in distinguishing between the action of enzymes and of possible contaminating bacteria in experimental work. Although this difference between enzymes and cells is characteristic, it does not finally decide that the cell actions are not enzyme actions, for it may well be that the poisons act chiefly by altering the physical conditions of the cell so that diffusion is interfered with, thus seriously interfering with the exchange of splitting products between different parts of the cell, and checking intracellular enzyme action, which we shall see later requires free diffusion of the products for its continuance.¹ At the very least, however, we may look upon the intracellular enzymes as the most important known agents of cell metabolism, and consequently of all life manifestations, and the changes they undergo or produce in pathological conditions must be fully as fundamentally important as is their relation to physiological processes. It therefore becomes necessary for us to consider carefully—

THE NATURE OF ENZYMES AND THEIR ACTIONS ²

Since up to the present time no ferment has been isolated in an absolutely pure condition we are entirely unfamiliar with

¹ Kaufman points out another important defect in the experiments indicating a difference between the effects of poisons on enzymes and on cells, namely, that in the experiments the concentration of enzymes is high, whereas in most cells it is low. Solutions of trypsin stronger than 0.2 per cent. are not much affected by toluol, thymol, etc., during twenty-four hours, but weaker solutions are—those less than 0.02 per cent. being rendered inert. (*Zeitschr. f. physiol. Chem.*, 1903 (39), 434.)

² It would not be profitable to discuss fully all the various theories and hypotheses that have been advanced, but the reader is referred to the following chief compilations of the entire subject: Oppenheimer, "Die Fermente und ihre Wirkungen," Leipzig, 1903, Efront, "Enzymes and their Applications,"

their chemical characters, and consequently are obliged to recognize them solely by their action. As far as we know, true enzymes never occur except as the result of cell life—they are produced within the cell, and increased in amount by each new cell that is formed, and, furthermore, they are present in every living cell without exception. As the same facts are equally true of the proteids, and apparently true of nothing else, it is natural to associate the enzymes with proteids, and so explain the importance of the proteids for cell life.¹ If enzymes are obtained in any of the usual ways from animal cells or secretions they are always found to give the reactions for proteids, even if repurified many times. But it is well known that whenever proteids are precipitated the other substances in the solution tend to be dragged down by the colloids, and it is possible that the enzymes are merely associated with the proteids in this way. Furthermore, enzymes are known to become so closely attached to stringy proteid masses, such as fibrin and silk, that they cannot be removed by washing. Some have claimed that they have secured active preparations of pepsin and invertase that did not give proteid reactions and contained very little or no ash or carbohydrate; but it has so far been impossible to secure trypsin free from proteid, and diastase seems to be certainly of proteid nature. Analyses of enzymes purified as completely as possible do not have great worth, for the “purified” enzymes are probably far from pure; however, it is of some importance that they vary greatly in the proportions of carbon, hydrogen, and nitrogen which they contain, indicating that possibly different enzymes may be of very different nature. Active gum enzymes, with oxidizing properties, are said to have been prepared free from nitrogen.² Macalium has shown microchemically that phosphorus is closely associated with the formation of zymogen granules in cells, which seem to be started in the nucleus; and there are many other observations suggesting that certain ferments are closely related to the nucleo-proteids. This is particularly true of the oxidases, which seem also to contain iron and manganese. A final point of importance in support of the proteid nature of

translated by S. C. Prescott, New York, 1902. Reynolds Green, “Soluble Ferments and Fermentation,” 1901. In this chapter references will not usually be cited unless they are from works published later than Oppenheimer’s book, in which all original work of importance can be found.

¹ Another important point is that the closest imitation of enzymes, Bredig’s “inorganic ferments,” seem to owe their action to their colloidal nature.

² A recent paper by Tschirsch and Stevens casts considerable doubt upon this statement (Pharmac. Centrally., 1905 (56), 501.)

enzymes is that pepsin destroys trypsin and diastase, while trypsin destroys pepsin.

So uncertain, however, is our information concerning the chemical nature of the enzymes, that it has become possible for a hypothesis to be developed urging that enzymes are immaterial, that the actions we consider as characterizing enzymes are the result of physical forces which may reside in many substances, and perhaps even free from visible matter. Arthus, who has been the chief champion of this very interesting conception, compares enzyme action to such forces as magnetism. A magnetic iron bar loses its characteristic property when sufficiently heated, just as an enzyme does. Dissolve the magnet or the enzyme in strong hydrochloric acid and they both lose their power to affect other substances. It has been equally impossible to isolate enzymes and magnetism, both of which are recognized by their actions, and not by themselves. Just as light, heat, and electricity were once considered as matter, so has it also been with enzymes, and Arthus believes that they will eventually be stricken from the list of material things and considered as forms or a form of energy. There can be no question that this conception rests on strong grounds, and it possesses the stimulating qualities that make a hypothesis helpful, but, as Oppenheimer says, all chemical substances may be considered in the same way. We recognize all bodies through some form of energy; if we speak of sulphuric acid, it is really of the properties of energy it shows, such as its taste, which is the energy imparted by its ions to the nervous system; or its combining with bases, etc., which also is a manifestation of energy. In the same way we recognize the ferments, and we may properly believe them to be fully as much definite substances as is sulphuric acid. The magnet comparison also falls when we remember that the magnetism can be introduced into a bar of iron and removed at will, but as yet it has not been possible to introduce enzymatic properties into an inert proteid, or to restore them to an enzyme that has been destroyed by heat.

Another valuable piece of evidence of the material existence of enzymes is their specific nature, lipase affecting only fats, and trypsin only proteids, indicating chemical individuality. They are true secretions, formed within the cell by recognizable steps; and, furthermore, when injected into the body of an animal, they give rise to the formation of specific immune bodies that antagonize their action. Emil Fischer's work with the sugar-splitting enzymes, moreover, indicates that they owe their

action to their stereochemical configuration. He prepared two sets of sugar derivatives which differed from each other solely in the arrangement of their atoms in space (*i. e.*, isomers) and found that one specific enzyme would split members of only one of the varieties, while another enzyme would act only on the variety with the opposite isomeric form. These experiments make it very probable that there must be a certain relation of geometrical structure between an enzyme and the substances it acts upon, and leaves little question of its material nature.

Bredig¹ has found that *colloidal solutions of metals* have many of the properties of true enzymes, accomplishing many of the decompositions produced by enzymes, being affected by temperatures of nearly the same degree, and even being "poisoned" by substances that destroy or check enzymes. The only possible explanation of these observations seems to be that the enzyme effects are brought about by *surface phenomena*. A colloidal solution of platinum, so far as is known, differs from a piece of metallic platinum solely in the enormously great amount of surface it offers in proportion to its weight, and it is well known that surfaces may affect chemical action. Hence we have the possibility that some enzyme actions, at least, may depend upon the existence of a very large surface, and since by no means all colloids are enzymes, that this surface must bear a certain relation in form to the surface of the body that is to be acted upon.

THE PRINCIPLES OF ENZYME ACTION

The effects produced by enzymes, which at one time were considered quite unique and remarkable, have now been made comparatively plain, chiefly through the observations of Ostwald on related chemical reactions; and by the investigations of Croft Hill, Kastle and Loevenhart, and others, on enzymes, which show that enzyme action is in no way different from chemical action observed independent of enzymes. The fundamental consideration is that chemical reactions are *reversible*, that is, that their tendency is to *establish an equilibrium*, and that the change may be from either side of the equation. The action of enzymes is similar to that of all catalytic agents, that is, they *increase the speed of reaction*. In the case of such a reaction as that of NaOH and HCl, the reaction is so rapid that the effect of catalyzers could hardly be noticed; but with

¹ Résumé in *Ergebnisse der Physiol.*, 1902 (Bd. I., Abt. 1), p. 134; also Bergell, *Zeit. klin. Med.*, 1905 (57), 382.

many other substances the reaction is very slow, and without the presence of catalyzers it would go on almost or quite imperceptibly. For example, ethyl butyrate saponifies on the addition of water according to the following equation :



On the other hand, if ethyl alcohol and butyric acid, the products of this reaction, are placed together, they will combine to form ethyl butyrate ; in other words, the reaction is reversible, as indicated by the arrows in the equation. In any event, however, the reaction is not complete, but continues only until a certain definite proportion of ethyl alcohol, butyric acid, ethyl butyrate, and water exists, when the change will stop, *i. e., equilibrium is established*. The time that would be required for this reaction to occur at room temperature would be extremely long, the change being hardly noticeable, but in the presence of a catalytic agent (which may be colloidal platinum or lipase) the reaction goes on much more rapidly. Catalytic agents, therefore, merely hasten reactions which would go on without them, and they do not initiate or change the nature of chemical reactions at all. When equilibrium is established, the reaction stops and the enzyme has nothing more to do. Furthermore, and this is a recently appreciated fact, enzymes will hasten synthesis just as well as they hasten catalysis. Croft Hill first showed that maltase would synthesize glucose into maltose ; Kastle and Loevenhart soon after established the synthesis of ethyl butyrate under the influence of lipase, and Neilson¹ demonstrated that platinum black had the same property. Taylor² first synthesized one of the normal body fats, triolein, by the action of lipase (from the castor-oil bean) upon oleic acid and glycerin. It may seem improbable at first sight that the synthesis of proteids can be accomplished by enzymes, as is the relatively very simple synthesis of carbohydrates and fats, but the improbability disappears when we recall the well-known fact that the products of proteid splitting in passage through the intestinal wall disappear and are reconverted either there or elsewhere into body proteids. Proteids manifestly are synthesized and we have not a little reason to believe that this is accomplished by enzymes, presumably by a reversal of their action in the establishment of equilibrium. Taylor was unable to synthesize protamin, one of the simplest proteids, by the action of trypsin upon its cleavage products, but it has been

¹ Amer. Jour. of Physiol., 1903 (10), 191.

² Univ. of California Publications (Pathology), 1904 (1), 33.

found that the addition of proteolytic enzymes to solutions of pure albumose leads to the formation of a jelly-like, insoluble proteid substance, which seems to be the effect of a reversed action on the part of the enzymes.¹ Indeed, the question has been raised whether the coagulating or "lab-ferment" (rennin) of the stomach is anything more than the pepsin itself, acting in a reverse direction.² Another well-known synthetic action that seems to be due to reversible ferment action is the formation of hippuric acid from benzoic acid and glycocholic acid in the kidney; the formation of glucose into glycogen and its reformation are also probably both accomplished by one and the same enzyme acting reversibly. Other reversible reactions less closely related to animal cells have also been described.

The reversible nature of enzyme action explains many problems of metabolism, and makes the whole field much clearer. The following consideration of the newer understanding of fat metabolism on this basis may explain the manner in which chemical changes are believed to occur in the cells and fluids of the body: ³

In the intestines fat is split by lipase into a mixture of fat, fatty acid, and glycerin; but as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine, so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Loevenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also appears, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to again overthrow chemical equilibrium, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that, as the composition of the contents of the intestines and of the blood-vessels varies, the direction of the enzyme action will also vary. In the blood-serum, and also in the lymphatic fluid, there is more lipase, which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.

Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood-serum bathing the cells is a mixture of fat and its

¹ Herzog, *Zeit. f. physiol. Chem.*, 1903 (39), 305.

² The results of filtration experiments suggest that pepsin and rennin are distinct substances (Levy, *Jour. Infect. Diseases*, 1905 (2), 1; also see Schmidt-Nielsen, *Zeit. physiol. Chem.*, 1906 (48), 92).

³ See Loevenhart, *Amer. Jour. of Physiol.*, 1902 (6), 331; Wells, *Journal Amer. Med. Assoc.*, 1902 (38), 220.

constituents, probably nearly in equilibrium, since lipase accompanies them. If the diffusible substances enter a cell containing lipase, *e. g.*, a liver cell, the process of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.

To summarize, it may be stated that throughout the body there is constantly taking place both splitting and building of fat. Fat enters the cells, leaves them, and is utilized only in the form of its acid and alcohol, never as the fat itself. Fat constitutes a resting stage in its own metabolism.

If proteolytic enzymes are also reversible, then the phenomena of proteid metabolism are similarly explained, for there is no doubt that every cell and body fluid contains proteolytic enzymes.

All metabolism, then, may be considered as a *continuous attempt at establishment of equilibrium by enzymes, perpetuated by prevention of attainment of actual equilibrium through destruction of some of the participating substances by oxidation or other chemical processes, or by removal from the cell or entrance into it of materials which overbalance one side of the equation.*

In just what manner the enzymes accomplish their catalytic effect is yet unknown. A favorite idea is that they form loose compounds with the body to be split and with water; the resulting compound being unstable and breaking down, the water remaining attached to the components of the substance. There is some evidence, but not conclusive, indicating that the enzyme does enter into combination with its object. Euler has suggested that enzymes increase ionization, which is at the bottom of the chemical changes.

Enzymes do not act catalytically on all substances by any means, but show a decidedly specific nature. They affect only organic substances, and the actions are limited to two processes—hydrolysis and oxidation, or the reverse processes of dehydration and reduction.¹ The most essential difference between the enzymes and the chemicals that can accomplish hydrolysis or oxidation is this: the ordinary chemical reagents produce their effects on many sorts of substance, the enzymes are specific; thus hydrochloric acid will hydrolyze starch or proteid with equal facility, but pepsin will not affect starch at all.

The very specific nature of the enzymes, their activation by other body products, the fact that they seem to be bound to the substance upon which they act, that they are susceptible to heat,

¹ Alcoholic fermentation may be an exception, the change being $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$, but it is very possibly an intramolecular oxidation.

and that they produce immune bodies when injected into experimental animals, all suggests the probability of a *relationship between enzymes and toxins*. This matter will be discussed more fully in considering the chemistry of immunity against enzymes.

General Properties of Enzymes.—Other properties of enzymes may be briefly mentioned. The speed of reaction they produce increases with the amount of enzyme present, but not in direct proportion (except with rennin). Very dilute acids favor the action of nearly all ferments, and alkalies are unfavorable for all but trypsin, ptyalin, and a few others. Weak salt solutions also are more favorable than distilled water. (These facts suggest strongly the possibility that *ions* play an important rôle in the process.) Water and dilute glycerin dissolve enzymes, which form always colloidal solutions that are very slightly dialyzable; and they may be precipitated from solution by alcohol, and redissolved again with but slight impairment of strength. Filtration through porcelain filters is not complete, from 10 to 25 per cent. of most enzymes being lost in each filtration.¹ As before mentioned, many chemicals poisonous to bacteria have little influence on most enzymes, but nearly all substances when concentrated are injurious or destructive, and some enzymes are known that are more susceptible to antiseptics than are the cells that contain them. Formaldehyde is very destructive to enzymes, even when dilute. The effect of proteid-coagulating antiseptics upon enzymes is, of course, greatly modified by the amount of proteid substances mingled with the enzymes; and the effects of heat and other injurious influences are greatly decreased by the presence of proteids and other impurities.

All enzymes are most active between 35° and 45° C., and it is interesting to note that Kobert² found this equally true for enzymes derived from cold-blooded animals. Although enzymes can stand temperatures of 100° C. or more when dry, in water they are generally destroyed somewhat below 70° C. Low temperature, even—190° C.,³ (liquid air), does not destroy them. The loss of power through heating disappears gradually, and there is no sharp line at which their action disappears. Sunlight is harmful to enzymes in solution, but only in the presence of oxygen⁴; this effect is augmented by the presence of fluorescent substances. Radium and *x*-rays seem to have a dele-

¹ Literature, see Levy, Jour. Infectious Diseases, 1905 (2), 1.

² Pflüger's Arch., 1903 (99), 116.

³ Bickel, Deut. med. Woch., 1905 (31), 1383.

⁴ Iodlbauer and Tappeiner, Deut. Arch. klin. Med., 1905 (85), 386.

terious effect upon most enzymes, and retard their rate of action ; but, apparently, autolytic enzymes (Neuberg¹) and tyrosinase (Willcock²) are not injured by these agencies. Labile as enzymes are, their persistence when dry is remarkable ; Kobert found active trypsin in the bodies of spiders that had been in the Nuremberg Museum for 150 years, and Sehrt³ found that the muscle tissue of mummies contained active glycolytic ferment.

All enzymes as ordinarily prepared have the property of decomposing hydrogen peroxide, a property possessed by substances of varied nature; this effect is prevented by CNH, which does not prevent other enzyme manifestations, indicating that this property is due to an associated enzyme, *catalase*.

The retardation of enzyme action by accumulation of the products of their action is simply explained as being due to establishment of equilibrium; in some instances, however, the substances produced are of themselves harmful to the enzymes, *e. g.*, alcohol and acetic acid.

Activation of Enzymes.—Within the cell, the enzymes—at least those that are excreted, such as trypsin and pepsin—exist with few exceptions in an inactive form, the *zymogen*. Their activation appears to take place normally only after they have been discharged from the cell, but after the death of an organ it may result from the decomposition products that are formed. Under physiological conditions this activation appears to be brought about by special activating substances. In the case of the pancreas it is the *enterokinase*, which is furnished by the epithelial cells of the intestine. Enterokinase appears to unite with trypsinogen to form an active enzyme, which reminds one of the way that complement and the intermediary body unite to form hemolytic and bacteriolytic substances.⁴ *Kinases*, having the same action as enterokinase upon the trypsinogen, are found in various tissues and organs, but generally much less active than the enterokinase. Pepsinogen is probably activated by the HCl of the gastric juice. A similar activating process seems to be essential for the production of the glycolytic ferment of the muscle and liver, the pancreas furnishing the activator in this case. It is very probable that it is through this mechanism

¹ Berl. klin. Woch., 1904 (41), 1081.

² Jour. of Physiol., 1906 (34), 207.

³ Berl. klin. Woch., 1904 (41), 497.

⁴ Bayliss and Starling (Jour. of Physiol., 1905 (32), 129), question the analogy of zymogen-kinase combinations to complement-amboceptor combination. Walker, however, finds evidence that many enzymes consist of a specific amboceptor and a non-specific complement or kinase (Jour. of Physiol., 1906 (33), p. xxi.).

that the rate of enzyme action is modified, and perhaps it is a means of defense of the body against its own enzymes; as the prozymes are more resistant to harmful agencies than the enzymes, it also may be a method of storage.

THE TOXICITY OF ENZYMES

Although present normally in greater or less amounts in all the cells in the body, when artificially isolated and injected directly into animals nearly all enzymes seem to be extremely toxic. The first thorough study of the toxicity of enzymes was made by Hildebrandt,¹ who found that pepsin, invertase, diastase, emulsin, myrosin, and rennin were all toxic. Emulsin and myrosin were most toxic, 0.05 gram being the lethal dose for a rabbit (average size); for pepsin, invertase, and diastase the lethal dose was 0.1 gram, while rennin required 2 grams. The symptoms produced in dogs were trembling, uneasiness, difficulty in walking, and finally coma. The anatomical changes observed were: numerous hemorrhages throughout the body, fatty degeneration of the liver and myocardium, renal congestion, and numerous thromboses. Considerable fever results, and Mayer considers this responsible for the relative harmlessness of rennin, the action of which is impaired above 40°. That these effects are due to the enzymes themselves rather than to contaminating bacteria is shown by Kionka and by Achalme² who obtained similar results with enzymes made sterile by filtration through porcelain. Achalme found that such sterile preparations of pancreatic juice injected subcutaneously into guinea-pigs produce a marked local pink gelatinous edema, followed by gangrene; if the animal dies, the blood is non-coagulable. Intraperitoneal injection is better withstood than subcutaneous. Fiquet³ also observed that trypsin and pepsin rendered the blood incoagulable, but after some time the coagulability of the blood is increased and thrombosis is frequent. Wells⁴ found that pancreatic extracts containing very active trypsin and lipase injected intraperitoneally produced an acute inflammatory reaction, but no fat necrosis. Extracts containing active lipase and inactive trypsin were less toxic, but produced fat necrosis. Extracts of liver and blood-serum, rich in lipase, were almost without effect on dogs and cats. *Papain* was found to be much more toxic than any animal

¹ Virchow's Archiv, 1890 (121), 1.

² Ann. d. l'Inst. Pasteur, 1901 (15), 737.

³ Arch. d. Méd. Exper., 1899 (11), 145.

⁴ Jour. Med. Research, 1903 (9), 92.

enzyme, causing violent local hemorrhagic inflammation. Schepilewsky¹ also found papain much more toxic than rennin and pancreatin; repeated injection of the two latter caused amyloidosis in rabbits. Lombroso² found that inactive pancreatic juice was much less toxic than the activated, showing that it is the trypsin that is the important toxic agent. He also found that *succus entericus* in doses of 1 to 5 c.c. is toxic, but not lethal for dogs. Hildebrandt³ observed that enzymes were positively chemotactic, but it is probable that the products of their action on the tissues are the chief chemotactic agents.

The enzymes that are secreted into the gastro-intestinal tract seem to be chiefly destroyed, but part is eliminated in the feces, and part that is absorbed apparently reappears in the urine in very small quantities. Pepsin, diastase, and rennin all have been found in normal urine; but the occurrence of trypsin is unsettled. Zeri⁴ could find no lipase in normal or nephritic urine, except when blood or leucocytes were present. Ferments injected subcutaneously seem seldom to be eliminated in any considerable amounts in the urine, but Opie⁵ has demonstrated the presence of lipase in the urine in pancreatitis with fat necrosis. Hildebrandt was able to prove that emulsin remained active for at least six hours after it was injected into animals subcutaneously, by its splitting amygdalin which was then injected, the CNH liberated by the cleavage of the amygdalin causing death.

Anti-enzymes.—Injection of enzymes into animals leads to the appearance of substances in the serum of the animals that antagonize the action of the enzymes. The principles involved are quite the same as in the immunization of animals against bacterial toxins or against foreign proteids. This seems to have been first observed by Hildebrandt, and it has been taken up extensively in recent years in the study of the problems of immunity. An interesting observation that was made rather early in these studies was that normal blood-serum possesses a marked resistance against the action of proteolytic enzymes, not being at all digested by dilutions of enzymes that will rapidly digest a serum that has been heated. This property seems to be shared by egg-white and by the tissues and organs of the body (Levene and Stookey⁶). The anti-enzyme action seems to be

¹ Cent. f. Bakt., 1899 (25), 849.

² Abstract in Biochem. Centralblatt, 1903 (1), 712.

³ Virchow's Arch., 1893 (131), 5.

⁴ Il Policlinico, 1905 (12), 733.

⁵ Johns Hopkins Hosp. Bull., 1902 (13), 117.

⁶ Jour. Medical Research, 1903 (10), 217.

easily destroyed, by heat of about 70° , by the action of dilute acids, and even by prolonged standing. It is exerted not only against the secreted proteolytic enzymes, pepsin and trypsin, but also against the intracellular enzymes of various organs.

It seems highly probable that the resistance of the body tissues to digestion by their own enzymes and by the enzymes of one another depends in some way upon the presence of anti-enzymes in the cells and tissue fluids. Weinland¹ has demonstrated that certain intestinal worms contain a strong antitrypsin, to which he attributes their ability to live bathed in pancreatic juice without being digested. Similar properties have been ascribed by other observers to the cells of the mucosa of the stomach² and intestine. An anti-catalase has been described as present in the tissues of the body by Battelli and Stern.³ The anti-enzymes seem only to inhibit enzyme action, and not to destroy the enzyme itself.⁴ Normal anti-enzymes do not seem to be at all specific, according to v. Eisler,⁵ that is, human serum is no more resistant to human trypsin than is pig serum—indeed, it is less so.⁶

Cathcart⁷ believes that antitrypsin is connected with the "albumin fraction" of the serum, *i. e.*, the fraction precipitated between half and full saturation with ammonium sulphate. Globulins do not possess this action, but they are not easily digested. He found antitrypsin in all varieties of serum, and considers it little or not at all specific. It is destroyed by 55°C .⁸ for one-half hour, but retains its anti-enzymatic activity after drying. The isolated body is equally effective against all sorts of proteids. Glaessner⁹ claims that the antitrypsin is united to the euglobulin fraction of the blood-serum, and that it is most abundant during periods of digestion. Fuld and Spiro¹⁰ found that the natural antirennin of normal horse

¹ Zeit. f. Biol., 1903 (44), 45; see also Dastre and Stassano, Compt. Rend. Soc. Biol., 1903 (55), 130 and 254; and Hamill, Jour. of Physiol., 1906 (33), 479.

² See Blum and Fuld, Zeit. klin. Med., 1906 (58), 505.

³ Jour. Phys. et Path. gén., 1905 (7), 919.

⁴ According to Delezenne and others, antitrypsin exerts its effects chiefly by combining with the kinase that activates trypsinogen, rather than with the trypsin. Bayliss and Starling (Jour. of Physiol., 1905 (32), 129) oppose the view of Delezenne that the antitryptic action of the blood is due to an antikinase, and believe the antibody acts upon trypsin.

⁵ Ber. d. Wien. Akad., 1905 (104), 119.

⁶ This is contradicted by Glaessner (*loc. cit.*).

⁷ Jour. of Physiol., 1904 (31), 497.

⁸ Unless otherwise specified, all temperatures are given according to the Centigrade scale.

⁹ Hofmeister's Beiträge, 1903 (4), 79.

¹⁰ Zeit. f. physiol. Chem., 1900 (31), 132.

serum is in the pseudoglobulin fraction. Since acids destroy the anti-enzyme property of the serum, it is not effective against pepsin-HCl mixtures. Against trypsin, however, it is very effective. Red corpuscles and living unicellular organisms are likewise resistant to trypsin, and normal serum also seems to contain an antirennin.¹

Oppenheimer and Aron² consider it probable that the resistance of normal serum to trypsin digestion depends upon the configuration of the proteid molecules, which perhaps, when in fresh, uninjured condition, present no suitable surfaces for attack by the ferment.

Opie³ has found that the serum of inflammatory exudates contains an anti-enzymatic substance, destroyed at 75°, and by acids.

Ascoli and Bezzola⁴ state that the antitryptic action of the blood is increased during pneumonia, which is probably the result of a self-immunization against the ferments liberated by the disintegrated leucocytes.⁵

The anti-enzymatic property obtained in the serum by injecting enzymes into animals differs from that normally present in the serum in many ways. It may be made much stronger than it ever is in normal serum, and against many varieties of enzymes for which an anti-enzyme does not naturally exist. Especially important is the fact that it is highly specific (v. Eisler); serum of an animal immunized against dog trypsin will show a much greater effect against dog trypsin than it does against trypsin from other animals. This fact permits us to distinguish between enzymes of apparently similar nature but of different origin, and proves that they have a structure at least in some respects different from one another, since they are combined by different antibodies. Artificial immune serum has been obtained against trypsin, pepsin, lipase, emulsin, autolytic enzymes, tyrosinase, urease, rennin, catalase, and fibrin ferment.⁶ By immunization against bacteria an immunity against their proteolytic enzymes is also obtained.⁷

¹ Czapek (Ber. Deut. botan. Gesell., 1903 (21), 229, states that anti-oxidases occur normally in certain plants, strongly specific against the oxidase of the same plant species.

² Hofmeister's Beiträge, 1903 (4), 279.

³ Jour. Exp. Med., 1905 (7), 316.

⁴ Berl. klin. Woch., 1903 (40), 391.

⁵ Beitzke and Neuberg (Virch. Arch., 1906 (183), 169) have suggested that anti-enzymes may act by causing a synthesis that opposes the catalysis of the enzyme.

⁶ For a review of much of the literature on this subject see Schütze, Deut. med. Woch., 1904 (30), 308.

⁷ v. Dungern, Münch. med. Wochenschr., 1898 (45), 1040.

Resemblances of Enzymes and Toxins.—As can be seen from the above statements, the enzymes behave in many respects like the toxins, both in their manner of acting upon other substances and in the reaction they produce when introduced into the bodies of animals. As Oppenheimer says, "the bonds between enzymes and toxins are drawing closer and closer." According to some experiments, the enzymes behave much as if they possessed a haptophore and a toxophore group, the former of which combines with the substance that is to be acted upon; and immunity appears to be produced by the development of receptors that combine the haptophore groups, these receptors constituting the antiferments. Korschun¹ has even succeeded in obtaining an anti-antirennin. He also secured rennin in an altered condition so that it did not coagulate milk, but still did unite with antirennin—a "*fermentoid*," according to the Ehrlich nomenclature. This is a strong piece of evidence of the similarity of enzymes and toxins. On the other hand, an important difference between the enzymes and the toxins is that toxins produce their effects according to the law of definite proportions, which is quite different from the behavior of catalyzing agents. Also some of the toxins have greater heat resistance than most enzymes, whereas complement is more easily destroyed than are the enzymes.²

THE INTRACELLULAR ENZYMES

Until a very recent time our knowledge of enzymes in the animal body was limited to those present in the digestive secretions. With few exceptions these are without influence in pathological processes, since they seem to be but little absorbed, and rarely enter the blood or tissue in any other way. But with the more recently disclosed intracellular enzymes, many of which are present in every cell, the relation to pathology is very intimate. These intracellular enzymes, as we now know them, and their chief properties, are as follows:

OXIDIZING ENZYMES

Although oxidation of organic compounds is the chief source of energy in the animal body, yet the way in which it is accomplished is very little understood. We only know that it is brought about within the cells,³ and that substances that out-

¹ Zeit. f. physiol. Chem., 1902 (36), 141; 1903 (37), 366.

² The supposed relationship of enzymes and toxins is questioned by Liebermann, Deut. med. Woch., 1905 (31), 1301.

³ Lillie (Amer. Jour. of Physiol., 1902 (7) 412, has demonstrated that oxidation occurs chiefly about or within the nucleus.

side the body are oxidized with difficulty, are completely oxidized to carbon dioxide and water within the cells, and that this is done with just such a degree of rapidity that the heat produced is in exactly the amount necessary for the wants of the body.¹ There can be little question that this oxidation is accomplished through catalytic agents acting within the cells, and certain of them have been placed in a condition permitting of study. As yet their exact relations to intracellular oxidation are not clearly defined, but for the present they may be grouped provisionally as oxidizing enzymes. One of the most studied of these is—

Catalase.—It has long been known that most enzymes possess the power of decomposing hydrogen peroxide, with liberation of oxygen; but it was not until 1901 that it was finally demonstrated by Loew² that this property was due to a separate enzyme and was independent of the specific properties of the various other enzymes.³ This ferment is very wide-spread, and so is generally obtained along with the other enzymes when attempts are made to isolate them from the cell. It was named *catalase* by Loew, and he described two forms, *α-catalase*, which seems to be a nucleoproteid, and *β-catalase*, which has the properties of an albumose. It has been demonstrated by Bach and Chodat⁴ that peroxides are contained in plant cells, and from the wide distribution of catalase it seems probable that they also occur in animal cells. Just what function the catalase performs is at present merely a matter of speculation. Loew considers that it destroys peroxides formed in metabolism, which are very poisonous to cell life. Shaffer⁵ has found evidence that under the influence of catalase the oxygen liberated is in the molecular form, O₂, and therefore relatively inert; whereas when peroxides spontaneously decompose, they liberate atomic oxygen which is an active oxidizing agent. He found that uric acid is oxidized by peroxide of hydrogen, but when catalase is present, this oxidation is prevented. According to this the function of catalase is rather to prevent dangerous forms of oxidation than to help in normal oxidative processes. For the present, however, nothing can be said positively on this subject.

¹ A full discussion of this subject is given by Hammarsten, "Physiological Chemistry," introductory chapter.

² Report No. 68, U. S. Dept. of Agriculture.

³ Other observers had previously suggested the same possibility, and Jacobson had proved the independence of catalase action.

⁴ Berichte der chem. Gesellsch., Vols. 35 and 36: several articles.

⁵ Amer. Jour. of Physiol., 1905 (14), 300.

Occurrence of Catalase under Normal and Pathological Conditions.—Battelli and Stern¹ in one of the most recent studies have found that the catalytic power of the tissues endures many hours after death. Its abundance is different for different organs of the same animal, but remarkably constant for the same organ in the same species. In general the order in decreasing strength is: liver, kidney, blood, spleen, gastro-intestinal mucosa, salivary glands, lung, pancreas, testicle, heart, muscle, brain; but this order varies in different species. In embryos catalase is scanty, but it increases rapidly after birth. Leucocytes contain little, most of that in the blood being in the stroma of the red blood-corpuscles. The body fluids contain little or none. Acute poisoning by phosphorus or CNH, icterus, and double nephrectomy do not reduce the amount in the tissues; in chronic phosphorus poisoning the amount of catalase in the degenerated liver is decreased, but it is increased in the other organs. Injected intravenously, catalase (of the liver) is destroyed rapidly, and does not appear in the urine; it does not cause any toxic effects, nor does it increase resistance to poisoning by venoms. The tissues also contain *anti-catalases*, and still further a substance which protects the catalase from the anti-catalase; this protective substance is called the *philocatalase* by Battelli and Stern.² Jolles and Oppenheimer³ devised methods for quantitative estimation of the catalase of the blood, and found that it might be considerably reduced in diseases (nephritis, tuberculosis, and carcinoma, but not in diabetes), but the results were quite inconstant in each condition. Carbon monoxide poisoning did not lower the catalase action, and there is no difference between arterial and venous blood in the amount of catalase. The catalase action is independent of the hemoglobin, and it is not responsible for the formation of oxyhemoglobin; it is much less abundant in amphibia than in man.

The gas evolved by the action of pus on H_2O_2 was found by Marshall⁴ to be pure oxygen, each c.c. of a certain sample of pus examined liberating 133.9 c.c. of gas. The active constituent of pus, he states, is contained in the serum and not in the corpuscles.

Substances decomposing H_2O_2 have been found also in bacterial cultures, first by Gottstein, and later in the cell-juices

¹ Archivio di Fisiologia, 1905 (2), 471. This article contains a complete résumé of the literature to date (in French).

² Jour. physiol. et path. gén., 1905 (7), 919 and 957.

³ Virchow's Arch., 1905 (180), 185.

⁴ Univ. of Penn. Med. Bull., 1902 (15), 366.

expressed from tubercle bacilli by Hahn. Loewenstein¹ found an enzyme agreeing with catalase in filtered bouillon cultures of diphtheria bacilli and staphylococci but not from tetanus, typhoid, and colon bacilli or cholera vibrios; the catalase is quite distinct from the toxin. He also found that the addition of H_2O_2 to a diphtheria toxin-antitoxin mixture destroyed the toxin, leaving the antitoxin free. A similar destruction of tetanus toxin by peroxides, first demonstrated by Sieber, can occur without the catalase.

True Oxidizing Enzymes.—While it is by no means certain that catalase is active in causing intracellular oxidations, there are a number of other enzymes or enzyme-like substances that come more properly under the head of oxidases or oxidizing enzyme. Those so far studied are:

Peroxidase.—This name is given to an enzyme that is believed to cause oxidation by activating peroxides, and is quite distinct from catalase and from the other oxidases. The peroxide on which they chiefly act in the cell is supposed by Bach and Chodat² to be the enzyme oxygenase.

Oxygenase.—This enzyme can also act as an oxidizer independent of the peroxidase, in the presence of certain manganese compounds. Loewenhardt and Kastle³ question the true enzyme nature of this and other "oxidases," which they look upon as organic peroxides, behaving like other peroxides rather than as catalyzers. Practically the knowledge of these bodies is demonstrated by their power to turn tincture of guaiac blue, and they are, therefore, present in pus.

By their conception of oxygenase and peroxidase Chodat and Bach would displace entirely the idea of enzymes oxidizing directly, the true "oxidases," which they consider mixtures of oxygenase and peroxidase. How far this is justifiable may well be questioned. There have been, in any event, a number of ferments described that seem to possess distinct oxidative powers. As each is quite specific in its action, oxidizing but one substance, or one group of related substances, they are generally designated by the name of the substances upon which they act. Most studied of these is—

Aldehydease, which is characterized by oxidizing aldehydes, particularly salicyl-aldehyde. According to Jacquet, this enzyme

¹ Wien. klin. Woch., 1903 (16), 1393.

² Biochem. Centralblatt, 1903 (1), 417 and 457, where is also given a résumé of the literature.

³ Amer. Chem. Jour., 1903 (29), 563.

is so intimately bound with the cell that it cannot be obtained in extracts until after the cells are dead, but is present in expressed cell-juices. It can be isolated by the usual methods, is destroyed by boiling, and its action is inhibited by CNH. It has been demonstrated in nearly all organs and tissues except pancreas, muscle, marrow, and mammary gland; it is present in the blood in small amounts, but not at all in the bile (Jacoby¹). It is most abundant in the liver and spleen, and is present in pig embryos, 9 cm. long, but not in those 2–3 cm. long. Jacoby has obtained a body with the properties of aldehyde which did not give proteid reactions. It is a true enzyme, since it oxidizes aldehydes without itself being used up. Its range of action is limited, for Jacoby found it without effect upon acetic acid and stearic acid.

Tyrosinase.—This enzyme, which is found both in animal and plant tissues, is particularly interesting in relation to the formation of pigments. Bertrand found that the transformation of the juice of lac-yielding plants into the black lacquer was brought about by the action of an oxidizing ferment, *laccase*, upon an easily oxidized substance, *laccol*, which is a member of the aromatic series. He later found in a number of plants an enzyme acting on tyrosin, distinct from the laccase, which he named *tyrosinase*. Biederman² later found tyrosinase in the intestinal fluid of meal worms. v. Fürth and Schneider³ found a similar enzyme in the hemolymph of insects and arthropods, which explains its darkening when exposed to air. This enzyme, as obtained from different sources, is not always specific for tyrosin, frequently oxidizing other substances. v. Fürth and Schneider found the product of oxidation of tyrosin by animal tyrosinase related to certain of the *melanins* of animal tissues, and believe that tyrosinase is responsible for the production of many normal pigments. In the ink-sacs of the squid, which eject an inky fluid containing melanin-like pigment, tyrosinase was also found, corroborating this hypothesis. Florence Durham⁴ suggests that tyrosinase in the skins of animals is responsible for their pigmentation.

Gonnermann⁵ found that tyrosinase from beet-root produced *homogentisic acid* by acting on tyrosin, which is of interest in connection with the congenital hereditary disease, *alkaptonuria*

¹ Ergebnisse der Physiol., 1902 (Bd. I, Abt. 1), p. 213, where is also given a résumé of the subject of intracellular enzymes.

² Pflüger's Arch., 1898 (72), 105.

³ Hofmeister's Beitr., 1901 (1), 229.

⁴ Proc. Royal Soc., 1904 (74), 310.

⁵ Pflüger's Arch., 1900 (82), 289.

(*q. v.*), in which the urine becomes dark upon exposure because of the presence of homogentisic acid.

Other Oxidizing Enzymes.—Of the great number of other less studied oxidizing enzymes little can be definitely stated. Some consider that they are largely different manifestations of the action of one oxidizing ferment, but against this view Jacoby mentions that they occur distributed unequally in different organs, can be separated from each other, and they cause different reactions. For the catalase and for laccase (which produces the Japanese lacquer by an oxidizing process) and perhaps for other oxidizing ferments, iron and manganese may be essential constituents. Bertrand¹ considers that laccase is an organic manganese compound.

Among these little known oxidizing ferments is one that seems to act specifically on the purin bases, changing them into uric acid (Spitzer²), and one which destroys uric acid, in the presence of catalase (Croftan³).

Reducing enzymes have not yet been satisfactorily demonstrated. It is possible that they do not exist, and that the intracellular reductions that are carried on within the cells are brought about by simple chemical reactions independent of catalysis, or it may well be that the oxidizing enzymes in some cases act reversibly; this possibility does not seem to have been investigated.

The best known intracellular reduction is that of methylene-blue, which can be readily studied experimentally because the blue color disappears on reduction of the dye. It is open to question if this particular reduction is due to a reducing enzyme. According to Ricketts⁴ the reduction depends upon two bodies, one thermostabile, the other thermolabile, recalling the reaction of complement and amboceptor. Johannsen⁵ found the liver most active in reducing methylene-blue, the kidney next. Extracts of the organs did not contain the reducing substance, which seems to be bound to the cell elements.

Oxidizing Enzymes in Pathological Processes.—Although the oxidizing enzymes undoubtedly play an important part in pathological conditions, they have been but little investigated from this standpoint. Jacoby found that they did not disappear from the degenerated liver in phosphorus poisoning

¹ Compt. Rend. Acad. Sci., 1897 (124), 1355.

² Pflüger's Arch., 1899 (76), 192.

³ Medical Record, 1903 (54), 6.

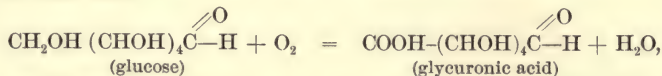
⁴ Jour. of Infectious Diseases, 1904 (1), 590.

⁵ Arb. aus d. path. Inst. Tübingen, 1905, vol. 5.

or in diabetes, or when the liver undergoes self-digestion, which speaks against Spitzer's contention that oxidase is a nucleoprotein.¹ Schlesinger² found that it is less in amount in livers of children dead from gastro-intestinal diseases than in normal livers, as also did Brüning.³ I am inclined to believe that fatty metamorphosis, when brought about by poisons, is often due to inhibition of the oxidizing enzymes (v. fatty metamorphosis). Buxton⁴ failed to find in *tumors* any enzyme giving the guaiac test alone, but found enzymes that did so in the presence of H_2O_2 (peroxidases). Catalase was present, but no very positive reactions for oxidizing enzymes were obtained by the indo-phenol reaction, the hydrochinon reaction, or with tyrosin for tyrosinase.

Meyer⁵ found that leucocytes, whether from pus or leukemic or pneumonic blood, contained a substance oxidizing guaiac directly, without the presence of H_2O_2 , which is not liberated until the cells are destroyed. The observation of Natalie Sieber⁶ that oxidases of the blood and of vegetable origin destroy diphtheria toxin rapidly, and also tetanus toxin and ricin, has been confirmed by Loewenstein as far as destruction by peroxide, with or without the presence of catalase, is concerned. Oxidation is undoubtedly an important process in defending the body against other forms of poisons. (See "Defense of the Body against Poisons of Known Composition.") Schmidt⁷ has found that by oxidation certain poisonous morphin derivatives are rendered non-poisonous by liver extracts. Oxalic acid and other poisonous fatty acids are also oxidized into harmless substances; phosphorus and sulphur are oxidized into their acids, which are then neutralized. Indol and skatol are oxidized into less harmful substances.

Glycolytic Enzymes.⁸—The oxidation of sugar by the tissues, which is one of the chief sources of energy in the animal body, presumably takes place through several steps. Of these, it is believed by some that the first is the formation of glycuronic acid—



¹ Duccheschi and Almagia (Arch. ital. Biol., 1903 (39), 29) also found the aldehydase in livers of phosphorus poisoning usually no less abundant than in normal livers.

² Hofmeister's Beitr., 1903 (4), 87.

³ Monat. f. Kinderheilk., 1903 (2), 129.

⁴ Jour. Med. Research, 1903 (9), 356.

⁵ Münch. med. Woch., 1903 (50), 1489.

⁶ Zeit. physiol. Chem., 1901 (32), 573.

⁷ Dissertation, Heidelberg, 1901.

⁸ Also discussed under "Diabetes," chap. xxii.

but the subsequent changes which involve decomposition of the straight chain are not at present understood. Alcohol and lactic acid are possibly steps in the process. Attempts to isolate from various organs an enzyme oxidizing glucose, particularly from the pancreas, muscle, and liver, have led to varying results and much dissention, but it is probable, because of these failures, that no such enzyme exists in quantities sufficient to account for the amount of sugar combustion that is normally accomplished. O. Cohnheim¹ seems to have explained the failures by his observation that the pancreas produces a substance that activates an inactive glycolytic enzyme in the muscles, liver, and probably in other organs. More or less of this activating substance or kinase is present in the blood and organs, determining a certain amount of activity in them when they are removed for experiment, and explaining the varying and inadequate amount of glycolysis often observed.

The activating substance is presumably an internal secretion from the islands of Langerhans, explaining the relation of these structures to diabetes. Cohnheim believes that the activator unites with the other components of the active enzyme, much as complement and intermediary body unite to form hemolytic and bacteriolytic substances. Although certain features of Cohnheim's work have been contested² the most essential features seem to be sufficiently confirmed; namely, that an interaction between extracts of the pancreas and extracts of muscle or liver produces much more glycolysis than the sum of their independent action would be.³

It is quite possible that the important enzyme in glycolysis is not an oxidizing enzyme, but that the ordinary oxidizing enzymes of the cell are able to attack the sugar only after it has first undergone a preliminary splitting by the specific "glycolytic" enzyme (O. Baumgarten⁴).

LIPASE

In all cells in which fat is found, and this includes practically all, lipase is probably present in greater or less amount. In the discussion of the reversible action of enzymes on a previous page the most modern conception of fat metabolism has been explained, which considers it to depend upon the existence of

¹ *Zeit. f. physiol. Chemie*, 1903 (39), 336.

² Claus and Emden, *Hofmeister's Beitr.*, 1905 (6), 214.

³ Simáček, *Cent. f. Physiol.*, 1903 (17), 477, and others have claimed priority, but Cohnheim's work was at least the first to attract general notice.

⁴ *Zeit. f. exp. Path. u. Ther.*, 1905 (2), 53.

lipase in the cells and fluids throughout the body. On account of the technical difficulties in the way of using the higher fats, such as triolein, in experimental work, the esters of lower fatty acids have generally been used, particularly ethyl butyrate. Enzymes splitting ethyl butyrate, and presumably higher fats, have been demonstrated in practically all tissues examined; the names of Kastle and Loevenhart in this country, and Hanriot in France, being particularly connected with this work. Whether in all cases the presence of this reaction is proof positive of the presence of an enzyme splitting fats, a true "lipase," is not yet known; undoubtedly as a rule it is, but there have been many claims made that true lipase does not exist in the blood-serum. From what is known about fat metabolism we have strong *a priori* grounds for believing that lipase is present in the blood-serum and in the lymph, and, also, we have some experimental evidence.¹

Little is known about the part played by lipase in pathological conditions. According to Achard and Clerc,² the amount of splitting of ethyl butyrate by the blood-serum is lessened in most diseases, and increases and decreases with the health of the patient. Clerc³ found that acute arsenic, phosphorus, and diphtheria-toxin poisoning increased this property of the serum while chronic poisoning and staphylococcus intoxication lowered it. Poulain⁴ found that the butyrate-splitting power of lymph-glands draining infected areas was decreased. It must be added that the value of these observations for considering pathological conditions is open to question. The same may be said of the reported finding of increased butyrate-splitting power in diabetic blood during diabetes with lipemia; Fischer⁵ observed, on the contrary, in a case of extreme lipemia in diabetes, that the lipolytic power of the blood was absent.

Lipase has also been demonstrated in pus by a number of observers,⁶ who agree that there is more in exudates than in transudates. Zeri⁷ found lipase in the urine only when pus or blood was also present.

The part played by lipase in fatty degeneration must be of great importance, but as yet it has been little considered, except

¹ Full references to the literature on lipase will be found in the article by Connstein (*Ergebnisse der Physiol.*, 1904 (Bd. 3, Abt. 1), 194).

² *Compt. Rend. Soc. Biol.*, 1902 (54), 1144.

³ *Compt. Rend. Soc. Biol.*, 1901 (53), 1131.

⁴ *Compt. Rend. Soc. Biol.*, 1901 (53), 786.

⁵ *Virchow's Arch.*, 1903 (172), 218.

⁶ Achalme, *Compt. Rend. Soc. Biol.*, 1899 (51), 568; Zeri, *Il Policlinico*, 1903 (10), 433; Memmi, *Clin. Med. Ital.*, 1905 (44), 129.

⁷ *Il Policlinico*, 1905 (12), 733.

that Duecheschi and Almagia¹ found no appreciable difference in the lipase content of normal and phosphorus-poisoned livers. This question will be considered more fully in discussing fatty metamorphosis.

Fat necrosis resulting from the escape of pancreatic juice into the peripancreatic tissues and abdominal cavity undoubtedly is largely the result of lipase action. Flexner² found lipase present in the foci of necrosis, and Opie demonstrated the escape of lipase into the urine in pancreatitis with fat necrosis. Wells (*loc. cit.*) was unable to produce fat necrosis with extracts of liver or blood-serum containing lipase, but found that pancreatic extracts rich in lipase produced fat necrosis, while the same extracts were ineffective after the lipase had been destroyed by the trypsin. (See "Fat Necrosis," Chap. xiii, for complete consideration.)

¹ Arch. Ital. Biol., 1903 (39), 29.

² Jour. Exper. Med., 1897 (2), 413.

CHAPTER III

ENZYMES (CONTINUED)

Intracellular Proteases¹ (Proteolytic Enzymes), Including a Consideration of Autolysis

To what extent synthesis of proteids goes on in the body is still a problem ; still more uncertain is the part played by reversible action of proteases. If the possibility of resynthesis of fats by lipase is still unsettled, the possibility of resynthesis of proteids by proteid-splitting enzymes must be still more open to question. There is evidence enough that somewhere in the body the amino-acids can be rebuilt into proteid, for Loewi,² and since him several others, has succeeded in keeping animals in nitrogenous equilibrium by feeding them products of proteolysis that contained no proteids whatever, and as the proteids of the animal body are incessantly being broken down, it must be that they were replaced by synthesis of the non-proteid material fed to the animals. In addition, it has long been known that amino-acids absorbed from the intestines do not reappear in the blood coming from the intestines, indicating that they are resynthesized into proteids while passing through the intestinal wall. Cohnheim³ found that in the intestinal epithelium there is an enzyme, *erepsin*, capable of splitting albumoses and peptones into the amino-acids, which enzyme presumably exists for the purpose of securing complete cleavage of all ingested proteids into their ultimate "building stones." This may be looked upon as a provision to reduce all varieties of proteids to their common elements, so that the body by quantitative selection can resynthesize them into its own types of proteid, for, as is well known, foreign proteids (*e. g.*, egg-albumin) introduced directly into the blood stream cannot be utilized, but are excreted unaltered in the urine. As was shown for lipase, the assumption that such synthesis occurs

¹ As long as the possibility still exists that ferments which digest proteids may be able to perform a certain amount of synthesis of proteids, the term "proteolytic enzyme" seems to be less suitable than the term "protease," which merely means an enzyme acting on proteids, and does not compel us to accept any particular view as to what the action is.

² Centr. f. Physiol., 1902 (15), 590.

³ Zeitschr. f. physiol. Chem., 1901 (33), 451 ; 1902 (35), 134.

as a normal physiological process by reverse enzyme action, requires that the proper enzymes be present in the cells throughout the body, and within the past few years it has been abundantly demonstrated that such is the case.

For over half a century it has been known that amebæ digest solid proteids within their bodies, but it is only within a few years that proteolytic enzymes have been definitely isolated from them. It has been much the same with the intracellular proteases of the higher organisms. In 1871 Hoppe-Seyler referred to the liquefaction of dead tissues within the body which occurred without putrefaction, and, as he noted, resembled the effects of the digestive ferments. In was nearly twenty years later that Salkowski¹ showed definitely that this softening of dead tissues was really brought about through a true digestion by intracellular enzymes, which produced the same splitting products that were at that time considered characteristic for tryptic digestion (leucin and tyrosin). The process he named "*autodigestion*." This important observation remained almost unnoticed for ten years more, when Jacoby,² in 1900, took up the investigation of this matter of cellular self-digestion, and after this the importance of the principles involved became for the first time generally appreciated. Jacoby rechristened the process "*autolysis*," by which name it is now commonly known.

AUTOLYSIS³

Autolysis is generally studied by the method used by Salkowski, which depends upon the difference in the susceptibility of bacteria and of enzymes to antiseptics. The organs are ground up to a pulp, placed in flasks with or without the addition of water or dilute acids, and bacterial action is prevented by the addition of antiseptics that are not poisonous to enzymes—toluol and chloroform are most commonly used. It is possible also to secure organs in an aseptic condition and to permit them to undergo autolysis without the use of antiseptics, but the practical difficulties are such that this method is seldom used—it is sometimes designated as "*aseptic autolysis*," in contradistinction to antiseptic autolysis by the Salkowski method. In a short time it can be seen that digestive changes have taken place, particularly if comparisons are made with control flasks in which the

¹ Zeit. f. klin. Med., 1890, supplement to Bd. 17, p. 77.

² Zeit. f. physiol. Chem., 1900 (30), 149.

³ Résumé of literature by Salkowski, Deutsche Klinik, 1903 (11), 147; also see Schlesinger, Hofmeister's Beiträge, 1903 (4), 87; Oswald, Biochem. Centr., 1905 (3), 365; Levene, Jour. Amer. Med. Assoc., 1906 (46), 776.

enzymes have been destroyed by boiling. To determine the rate of autolysis the amount of nitrogen that remains in the form of coagulable compounds, and that which is converted into soluble, non-coagulable compounds (albumoses, peptones, ammonia compounds, amino-acids, etc.), is compared. The method may be illustrated by a concrete example: A given specimen of emulsionized liver tissue was permitted to digest itself for twenty-two days. At the end of that time 39.4 per cent. of the nitrogen was still contained in the compounds that remained insoluble or became so after the autolysis was stopped by boiling; while 60.6 per cent. of the nitrogen was in a soluble form. A control specimen from the same liver was boiled while fresh to kill the enzymes, and then let stand under the same conditions. In this specimen 90.4 per cent. of the nitrogen was in an insoluble form, and 9.6 per cent. was soluble. Therefore, over half of all the proteid of the liver had been changed into non-coagulable substances in the course of about three weeks (at 37°C.).

Since Jacoby's paper appeared, the field has been invaded by many workers, who have examined practically every tissue in the body, and found that all possess the power of self-digestion; or, in other words, *proteases are present in every cell in the body*. The rate of digestion is very different in different organs, however, liver digesting rapidly while brain and muscle tissue digest much more slowly. These intracellular proteases are not altogether like either pepsin or trypsin, for they split proteids to its simplest elements, whereas pepsin carries the digestion only to the peptone stage (under ordinary conditions) and unlike trypsin their action is most marked in a faintly acid medium, and is entirely checked by alkalies no stronger than 0.4 per cent. NaOH, according to Wiener.¹ Furthermore, the cleavage products seem to contain a much larger proportion of the nitrogen in the form of ammonia and its compounds than is the case with tryptic digestion. It is quite probable that in autolysis several intracellular enzymes are in action, some of which may not be present in pancreatic or gastric juice. On the whole, however, the products are quite similar to those obtained by tryptic digestion. To give a concrete example, Dakin² detected in the products of autolysis by the kidney in acid solution, the following substances: Ammonia, alanin, α -aminovalerianic acid, leucin, α -pyrrolidin carboxylic acid, phenylalanin, tyrosin, lysin, histidin, cystin, hypoxanthin, and indol derivatives, including probably tryptophan.

¹ Centr. f. Physiol., 1905 (19), 349.

² Jour. of Physiology, 903 (30), 84.

During autolysis the changes are by no means limited to the proteids. Glycogen is split into glucose very early, and the sugar undergoes further changes. Fats are also split by the lipase, fatty acids being found in autolyzed organs. Reducing substances appear, and, as before mentioned, numerous volatile fatty acids are produced. The increase in fat described by some authors is probably only apparent, and due rather to the liberation of the fat from its combination with the proteids so that it is free and not "masked," as in normal organs. Lecithin is also decomposed, yielding cholin.

The *nucleo-proteids* seem to be attacked by the autolytic enzymes, as the purin bases are prominent among the products of autolysis, and in quite different proportions from those obtaining in digestion of the same tissues by other means. Apparently autolytic enzymes, like trypsin, attack the proteid group of the nucleoproteids, liberating the nucleic acids. These in turn are attacked by specific enzymes, *nucleases*,¹ which liberate the purin bases, which are further decomposed by specific enzymes, *guanase*, *adenase*, etc.²

It is improbable that the intracellular enzymes are merely pancreatic enzymes taken out of the blood by the cells, because of the differences previously cited; furthermore, Matthes³ found that the liver retained its autolytic power after the pancreas had been extirpated (in dogs), and that the autolytic degeneration of cut peripheral nerves went on just the same, indicating that the autolytic enzymes do not owe their origin to the pancreas.

RELATION OF AUTOLYSIS TO METABOLISM

It having been shown that proteases are present in all cells, the next question to be considered is, do they act only to destroy tissues after death, or are they of importance in metabolism? Since it is presumably necessary for proteids to be split into diffusible and easily oxidized forms in order that they may enter the cell, and be built up into the cell proteids, or be decomposed with the liberation of energy, the autolytic proteases may be assumed to be of prime importance in proteid metabolism; but to prove it is another matter. Jacoby found that if he ligated off a portion of the liver and let it remain *in situ* in the animal the necrotic tissues showed an accumulation of leucin, tyrosin,

¹ Sachs, *Zeit. physiol. Chem.*, 1905 (46), 337; Jones, *Ibid.*, 1903 (41), 101, and 1906 (48), 110.

² See Schittenhelm, *Ibid.* (42), 251; (43), 228; (46), 354.

³ *Arch. f. exp. Path. u. Pharm.*, 1904 (51), 442.

and other splitting products of the proteids, which suggested that these same bodies are being formed in the liver constantly, but that they are as constantly removed from the normal organs by the circulating blood, or are undergoing further alterations which cease when the circulation is checked. Among other observations possibly bearing on the same question are those of Hildebrandt,¹ who found that autolysis in the functioning mammary gland is much more active than in the resting gland; and of Schlesinger,² who found that autolysis was at its maximum (in rabbits) in new-born animals, decreasing rapidly in the first few months of life, and that in conditions associated with emaciation the rate of autolysis varied directly with the degree of emaciation. Wells³ sought for a possible influence on autolysis by thyroid extract, which increases proteid metabolism, but could demonstrate none *in vitro*. Schryver,⁴ however, found that autolysis was more rapid in the liver of dogs fed thyroid extract for some days before death than it was in control animals.

The possibility of synthesis of proteids by the autolytic enzymes seems not to have been investigated. Proteid synthesis seems to be accomplished on a large scale in the wall of the intestine, and the enzyme most prominent in this tissue is the *erepsin* of Cohnheim. In this connection the statement of Vernon⁵ that erepsin or a similar enzyme is present in all the tissues of the body may be of some significance. Erepsin is very similar to the autolytic enzymes, except that it does not attack proteids until they have been already split as far as proteoses, and the products of its action are not quite the same (Cohnheim). As yet the exact relation of erepsin to synthesis is quite unknown. The chief positive evidence yet obtained concerning proteid synthesis by proteases is the "*plastein* reaction," *i. e.*, the formation of an insoluble plastein when proteases are added to proteose solution; this occurs not only with trypsin and pepsin, but also with extracts of organs containing autolytic proteases.⁶

DEFENSE OF THE CELLS AGAINST THEIR AUTOLYTIC ENZYMES

The question of why the autolytic ferments do not destroy the cells until after death is a revival of the old problem of

¹ Hofmeister's Beiträge, 1904 (5), 463.

² *Ibid.*, 1903 (4), 87.

³ Amer. Jour. of Physiol., 1904 (11), 351.

⁴ Jour. of Physiol., 1905 (32), 159.

⁵ Jour. of Physiol., 1904 (32), 33.

⁶ Nürnberg, Hofmeister's Beitr., 1903 (4), 543.

"why the stomach does not digest itself," and the answer that satisfies some is that dead protoplasm is essentially different from living protoplasm. More exact replies are suggested by Wiener's studies on the relation of the reaction of the tissues to their autolysis. He found that autolysis does not begin in an organ until the original alkalinity is neutralized by the acids which are formed in all dead and dying cells.¹ If enough alkali is added to the material from time to time to neutralize the acidity as it develops, autolysis does not take place. Abundant amounts of organic acids are formed in autolysis of the tissues, principally lactic, acetic, and butyric (Magnus-Levy),² and the latent period between the time of the removal of an organ from the body and the appearance of autolysis may be explained by the time required for the neutralization of alkalescence. The old observation that rigor mortis disappears most rapidly in muscles that have been exhausted just before death is also probably explained by the greater amount of acid in such muscles.³ If we imagine that autolysis is limited to periods when the cells have an acid reaction, however, we limit their range of usefulness in the living cell to a minimum, since during life the tissue fluids, and presumably the cell contents, are preponderatingly alkaline. Perhaps a better explanation of the attack of the cells by their own enzymes after death is to be sought in the conditions of chemical equilibrium. During life constant new supplies of proteid are being brought to the cell, and at the same time the products of proteolysis are presumably being carried away by the circulation or being changed by oxidative processes. When circulation stops, the processes of splitting go on without the introduction of new supplies of material, and hence the tissues are not replaced as fast as they are destroyed, and the products of their decomposition accumulate, for lack of any means of destroying or removing them.

Still another possible defense of the living cells may be found in the existence of *specific antienzymes*. Just as the serum contains antitrypsin, so it seems to contain substances antagonistic to the autolytic enzymes. Levene and Stookey⁴ found that tissue juices show a resistance to digestion, and Opie⁵ found that the serum of inflammatory exudates retarded the

¹ Opie (*loc. cit.*) found, however, that autolysis of leucocytes was more rapid in an alkaline medium.

² Hofmeister's Beitr., 1902 (2), 261.

³ Delrez (Arch. internat. de Physiol., 1904 (1), 152) found by cryoscopic methods that muscle undergoes rapid autolysis during the first seven to nine hours after its removal from the body; after this the rate is much slower.

⁴ Jour. Med. Research., 1903 (10), 217.

⁵ Jour. of Exp. Med., 1905 (7), 316.

action of the autolytic enzymes that were contained within the leucocytes, and it is possible that continuance of the circulation may provide antibodies to the tissues to hold the intracellular enzymes in check, possibly without interfering with their action on other proteids than those of the cell structure.

There can be no question that the supply of food-stuff is of essential importance in determining autolytic changes, for it has been found by Conradi,¹ Rettger,² and Effront³ that bacteria and yeasts begin to undergo autolysis when they are placed in distilled water or salt solution, which they do not do, to any such extent at least, when in nutrient media. (In this way it has been found possible to obtain the intracellular toxins of such bacteria as typhoid and cholera.) Autolysis is not marked so long as the bacteria are supplied with nourishment, but when nutrient material is lacking, autolytic decomposition is no longer repaired and the bacteria disintegrate. Presumably the changes are the same in living cells, and anemic necrosis may be explained in this way. Tissue enzymes are also capable of digesting bacteria (Turró⁴).

Another direction in which the key to the action of these enzymes may be sought has been indicated by Jacoby,⁵ who found that to a certain degree the autolytic enzymes of each organ are specific for that organ. Liver extract will not split lung tissue, although it will split the proteoses that are formed in lung autolysis, possibly because these proteoses are less specific than the proteids from which they arise, or perhaps because of the erepsin the extract contains (Vernon). Leucocytic proteases, however, seem capable of splitting foreign proteids of all sorts. Richet⁶ states that the protease of liver tissue does not attack either muscle tissue or liver tissue that has been coagulated.

Lastly, it must be considered that at least to some extent the enzymes exist in the cells in their inactive *zymogen* form, which perhaps are changed into the active form as needed, and inhibited or changed back again when their work is temporarily finished. A rhythmical change of this nature might be imagined as occurring and accounting for interaction by the enzymes, particularly since rhythmical changes in metabolism are known to occur (*e. g.*) rhythmical production of carbon dioxide (Lyon⁷).

¹ Deut. med. Woch., 1903 (29), 26.

² Jour. Med. Research, 1904 (13), 79.

³ Bull. Soc. Chim., 1905 (33), 847.

⁴ Cent. f. Bakt., 1902 (32), 105.

⁵ Hofmeister's Beitr., 1903 (3), 446.

⁶ Compt. Rend. Soc. Biol., 1903 (55), 656.

⁷ Science, 1904 (19), 350.

AUTOLYSIS IN PATHOLOGICAL PROCESSES

All absorption of dead or injured tissues, and of organic foreign bodies, seems to be accomplished by means of digestion by the enzymes of the cells and tissue fluids. We may distinguish between the digestion brought about by the enzymes of the digested tissue itself, or *autolysis*, and digestion by enzymes from other cells or tissue fluids, or *heterolysis* (Jacoby). Heterolysis is accomplished particularly by the leucocytes, which contain ferments capable of digesting not only leucocytic proteids but apparently every other sort,¹ from serum-albumin to catgut ligatures. The heterolysis may be intracellular when the material to be digested has first been taken up by the cells (phagocytosis); or extra-cellular, either by enzymes normally contained in the blood plasma and tissue fluids, or by enzymes liberated by the leucocytes and fixed tissue cells. On death and dissolution of a cell the intracellular enzymes are released, but it is not known to what extent the enzymes may be secreted from intact living cells. As far as pathological processes show, the amount of liberation of enzymes from normal cells is very slight, if any, and the digestive enzymes of the blood plasma seem to be very feeble, but this is perhaps because they are largely held in check by the anti-enzymatic substances of the serum. Pathological autolysis and heterolysis, therefore, are brought about chiefly by enzymes liberated from dead or injured cells. Bacteria, however, can multiply upon a medium of coagulated proteid, which suggests that they also secrete proteolytic substances, for otherwise it would be difficult to explain how they secure their nourishment. In pathological conditions, digestion of degenerated tissues seems usually to be the result of both autolysis and heterolysis. An infarct softens because the intracellular enzymes digest the dead cells, exactly as they do when the tissue is removed from the body, ground up, and put in the incubator under toluol. In addition leucocytes wander in, disintegrate, and their liberated enzymes help in the process, as also do to a less degree the enzymes of the blood plasma. It is because of the heterolysis by leucocytic enzymes that a septic infarct becomes softened so much more rapidly

¹ Many authors suggest that the leucocytes merely carry enzymes from one organ, particularly the pancreas, to another, and that these enzymes are not formed by the leucocyte itself. Opie (Jour. Exp. Med., 1905 (7), 759) has shown, however, that the bone-marrow contains proteolytic enzymes which are like those of the leucocytes in that they act best in an alkaline medium, whereas the autolytic enzymes of the lymphatic glands and most other tissues act best in an acid medium. This leaves little room for doubt that the leucocytes are equipped with their characteristic enzymes when they leave the bone-marrow, and that they are not obtained later in the pancreas or elsewhere.

than does a sterile infarct, and by comparing the rate of softening in septic and aseptic infarcts we see that the cellular autolysis is a very slow process as compared to the heterolysis accomplished by the leucocytes. The explanation of this may lie in the fact that most intracellular proteases act best in an acid medium (Wiener), while leucocytic proteases act best in an alkaline medium (Opie), and the infarcts of small size are seeped through by alkaline blood fluids. When an infarct is large, we find it undergoing central softening while the periphery remains firm; this corroborates our hypothesis, for acids are developed during autolysis (Magnus-Levy), which at the periphery are neutralized by the blood plasma, so that only at the center is autolysis active. The inhibiting action of the serum also has a similar effect, limiting autolysis at the periphery.

In the case of septic softening the action of the bacteria needs also to be taken into consideration, since they also produce proteolytic ferments, but their effect seems to be relatively small as compared with leucocytic digestion. Intracellular digestion of necrotic tissue by leucocytes seems also to be relatively unimportant. Suppuration, therefore, must be considered as the result of digestion of dead tissue by enzymes derived from the leucocytes, the plasma, the bacteria, and the destroyed cells themselves. A tubercle does not ordinarily suppurate, because the tubercle bacillus and the substances it produces are not strongly chemotactic, and hence not enough leucocytes enter the necrotic area to produce a digestive softening. The enzymes of staphylococcus are much more strongly proteolytic than those of streptococcus (Knapp¹), which may be one reason why the latter so much more frequently produces lesions without suppuration than does the former. Necrotic areas of any kind are absorbed by similar processes. Autolysis of tumors is quite active in specimens removed from the body, and the areas of necrosis that occur commonly in tumors are absorbed in this way. Apparently all varieties of cells are subject to autolysis or heterolysis whenever they are killed or sufficiently injured. Atrophy may be looked upon as an autolysis in the normal course of catabolism, not met by a corresponding building up of the proteids.

The products of autolysis may of themselves be toxic; albumoses and peptones certainly are, and the other cleavage products are probably not altogether innocuous. (See "Autointoxication.") Some of the symptoms of suppuration, particularly the fever and chills, have been ascribed to the autolytic products rather

¹ *Zeit. f. Heilk. (Chir.)*, 1902 (23), 236.

than to the bacterial poisons, particularly as aseptic suppuration is accompanied by fever. Degenerative changes in nervous tissue are associated with autolytic decomposition of the lecithin (Noll¹) and the liberated cholin, or its more toxic derivatives, may be a source of intoxication.² In all conditions associated with autolysis, such as resolving pneumonic exudates, large abscesses, softening tumors, etc., albumoses (and peptones?) may appear in the urine. Autolytic products may also be hemolytic (Levaditi³), and they may prevent clotting of the blood (Conradi⁴).

Work has been reported upon autolytic processes in a number of pathological conditions, which may be discussed briefly as follows:

Exudates.—The presence of leucin, tyrosin, proteoses, and peptones in pus has been known for many years, and the reason for their appearance is now clear. Müller,⁵ many years ago, observed that purulent sputum digested fibrin, but that non-purulent sputum did not have this property. Achalme⁶ found that pus would dissolve gelatine, fibrin, and egg-albumen. Ascoli and Mareschi⁷ detected autolysis in sterile exudates obtained experimentally. Umber⁸ found that ascitic fluid exhibited autolytic changes, which observation could not be confirmed by Schütz⁹ in pleural exudates and ascitic fluids. Zak¹⁰ found that autolysis was inconstant in various exudates. The differences in these results are probably explained by Opie's¹¹ observation that in experimental inflammatory exudates the leucocytes are capable of marked autolysis, whereas the serum contains an antibody which holds this autolysis in check; if the antibody is destroyed by heat, then the serum proteids are also digested by the leucocytic enzymes. This antibody seems to be contained normally in the blood-serum. In old exudates the antibodies are decreased, and autolysis then occurs, explaining the variable results of Umber, Schütz and Zak. The intracellular proteases of the polynuclear leucocytes act best in an alkaline medium; those of the mononuclears in an acid medium. Exudates pro-

¹ Zeit. physiol. Chemie, 1899 (27), 380.

² See Halliburton, *Ergebnisse der Physiol.*, 1904 (4), 24.

³ Ann. d. l' Inst. Pasteur, 1903 (17), 187.

⁴ Hofmeister's Beitr., 1901 (1), 136.

⁵ Kossel, Zeit. f. klin. Med., 1888 (13), 149.

⁶ Compt. Rend. Soc. Biol., 1899 (51), 568.

⁷ See Maly's Jahresbericht, 1902 (32), 568.

⁸ Münch. med. Woch., 1902 (49), 1169.

⁹ Cent. f. inn. Med., 1902 (23), 1161.

¹⁰ Wien. klin. Woch., 1905 (18), 376.

¹¹ Jour. of Exper. Med., 1905 (7), 316 and 759; 1906 (8), 410.

duced by bacterial infection also seem to possess the properties above described. Galdi¹ found autolysis greater in exudates than in transudates, but observed no constant relation between the number of leucocytes, or the amount of chlorides, and the rate of autolysis.

Knapp² holds that in pus the cocci and the enzymes they produce are responsible for much of the digestion. Pus cells alone do not undergo digestion so rapidly as when bacteria are present, and digestion is more rapid if the bacteria are alive than when inhibited or killed by antiseptics. Streptococcus is almost inactive, staphylococcus is quite active, and *B. coli* still more so. He could find no relation between the autolytic power of the pus and the severity of the infection from which it resulted. (See also the discussion of the "Chemistry of Pus," Chap. x.)

Pneumonia.—In the stage of resolution lobar pneumonia presents a striking example of autolysis. The often-remarked phenomenon that the lung tissue itself is not in the least affected, while the dense contents of the alveoli are rapidly dissolved and removed is explained by the invariable immunity of living cells to digestive enzymes. Except for some slight possible assistance by the alveolar epithelium and the enzymes of the serum, the enormous and rapid digestion of pneumonic exudates is accomplished by the leucocytic enzymes. The rapid rate of digestion may be accounted for by the absence of circulation within the alveolar contents, which permits the leucocytes to act unimpeded by the anti-bodies of the blood plasma. Digestion of the exudate continues after death, accounting for the marked diffuse softening observed in pneumonic lungs in bodies kept some days before autopsy. As long ago as 1888, Kossel³ mentioned that Fr. Müller had found that glycerin extracts of purulent sputum exhibited a digestive action upon fibrin and coagulated proteid, whereas non-purulent sputum did not possess this property. In 1877 Filehne extracted ferments in the same way from the sputum in gangrene of the lung; Stolniknow in 1878, found a similar ferment in pneumonic sputa, and Escherich in 1885 showed that the proteolytic action of tuberculous sputum was independent of putrefaction. Other early observations of similar nature are reviewed by Simon,⁴ who demonstrated the presence of leucin and tyrosin in the autolyzed lungs. In a later work Müller reports finding three grams of leucin and

¹ See *Folia Hemat.*, 1905 (2), 529.

² *Zeitschr. f. Heilk.*, 1902 (23, Chir. Abt.), 236.

³ *Zeit. f. klin. Med.*, 1888 (13), 149.

⁴ *Deut. Arch. klin. Med.*, 1901 (70), 604.

tyrosin in a pneumonic lung, as well as lysin, histidin, and purin bases from the decomposed nucleoproteids. Flexner¹ noted that autolysis, while very rapid in the gray stage, is but slight in the red stage (because of paucity of leucocytes) and also in unresolved pneumonia, which he considers as due to some interference with autolysis. Silvestrini² found that in gray hepatization the reaction was strongly acid, in red faintly so; the gray hepatization showed more peptone, and leucin and lactic acid were both demonstrable. A fibrin-digesting enzyme was isolated, and milk was coagulated. Rzentkowski³ found an increase of non-coagulable nitrogen in the blood of pneumonics, probably resulting from autolysis in the exudate.⁴

Necrotic Areas.—Jacoby⁵ found that if a portion of a dog's liver was ligated off and the animal kept alive for some time the necrotic tissue contained the same products that he had obtained in experimental autolysis. The absorption of necrotic tissues generally is ascribable to either autolysis or heterolysis. Presumably there is no great difference in the self-digestion of an organ which is necrotic because its blood supply is cut off and of a similar organ removed from the body aseptically and allowed to undergo aseptic autolysis in an incubator. At the periphery there might be some effects produced by the inhibitive action of the serum or the digestive action of the leucocytes, but beyond that no marked differences are to be expected.

A study of the relation of autolysis to the histological changes that occur in necrotic areas by Wells⁶ gave evidence that there occurs early a decomposition of the nucleoproteids of the nuclei, which is probably brought about by the intracellular autolytic enzymes. The liberation of the nucleic acid and the reduction in the bulk of nuclear material through the digestion away of the proteid is probably the cause of the *pycnosis* observed in necrotic areas. Later the nucleic acids are further decomposed through the special enzymes described by Jones, Sachs, and others the "nucleases." This is presumably the cause of the loss of nuclear staining so characteristic of necrosis. That these changes are due to the intracellular enzymes was shown by implanting in animals pieces of sterile tissues, the enzymes of which had been destroyed by heating; these

¹ Univ. of Penn. Med. Bull., 1903 (16), 185.

² Bull. del Soc. Eustachiana, 1903, abstr. in Biochem. Centralbl., 1903 (1), 713.

³ Virchow's Arch., 1905 (179), 405.

⁴ Rietschel and Langstein (Biochem. Zeitschr., 1906 (1), 75), report the isolation of considerable quantities of leucin from the urine of a pneumonic.

⁵ Zeit. physiol. Chem., 1900 (30), 149.

⁶ Jour. Med. Research, 1906 (15), 149.

were found to undergo alterations only after several weeks, and then as the result of the action upon them of invading leucocytes. The slow rate of autolysis that occurs in infarcts and other aseptic areas is presumably due to the action of the antibodies of the serum, for it was found, experimentally, that the histological changes of autolysis when the tissues are placed in heated serum proceed about twice as rapidly as when they are placed in fresh serum. Chemotactic substances do not seem to be formed in aseptic dead tissues, but the slow absorption of such tissues is, however, finally accomplished by the leucocytes acting from the periphery, there being little actual autolysis of the dead cells by their own enzymes. The rapidity with which autolytic changes occur in different organs, as indicated by the disappearance of nuclear staining, seems to be about as follows: (1) Liver, kidney (epithelium of convoluted tubules); (2) spleen, pancreas; (3) kidney (collecting tubules, straight tubules, glomerules); (4) lung (alveolar and bronchial epithelium); (5) thyroid; (6) myocardium; (7) voluntary muscle; (8) skin (epithelium); (9) brain (cortical cells). Stroma cells seem to be attacked chiefly by enzymes from the parenchyma cells. Of all cellular elements, the endothelium of the vessels seems to have the greatest resistance to both autolysis and heterolysis.

Degenerated nervous tissue also undergoes a slow autolysis which, according to Noll,¹ results in the splitting of protagon with liberation of lecithin. Mott, Halliburton,² Donath, and others have shown that in nerve destruction lecithin is split up with liberation of cholin (see "Cholin"). Koch and Goodson³ found that degenerated nervous tissue is characterized, chemically, by containing a relatively increased amount of nucleoproteids, with an absolute decrease in solid constituents, while the lecithans are greatly altered.

In *caseation* autolysis is very slight, as is shown by the persistence of the caseous material for long periods of time without absorption. Presumably the toxin of tuberculosis destroys the autolytic ferments of the cells it kills, and as there is little chemotactic influence, leucocytes do not enter the caseous area. Spiethoff⁴ found that pure caseous material is usually free from even traces of albumose and peptone, but the caseous material at the periphery mixed with tissue elements contains them in very small quantities, suggesting that at the periphery of

¹ Zeit. physiol. Chem., 1899 (27), 390.

² General résumé in Ergebnisse der Physiol., 1904 (4), 24.

³ Amer. Jour. Physiol., 1906 (15), 272.

⁴ Cent. f. inn. Med., 1904 (25), 481.

caseous areas some slight autolysis does occur. The fact that *B. tuberculosis* is, itself, very poor in proteolytic enzymes as compared with most other bacteria may be another factor. When leucocytes are attracted into a tuberculous focus then softening goes on rapidly, showing that there is no loss of digestibility of the caseous material, but merely a lack of enzymes. Pus from a cold tuberculous abscess will not digest fibrin, but if iodoform is injected, leucocytes enter in great numbers, softening is rapid, and the pus will then digest fibrin (Heile¹).

Liver Degenerations.—The relation of the disintegration observed in *phosphorus-poisoning* and *acute yellow atrophy* to the experimental autolysis of the liver has been the object of much study. Salkowski originally pointed out that the same products were found in the blood, urine, and liver tissue in acute yellow atrophy as are produced in autolysis. Jacoby² found that the livers of dogs, taken just as the animals were dying of phosphorus-poisoning, contained free leucin and tyrosin; also, he found that the rate of autolysis of such livers after removal from the body was much greater than in normal livers. The oxidizing ferments (aldehydase) are not destroyed by the process. He found that addition of minute amounts of phosphorus to liver enzymes did not increase their proteolytic power; nevertheless, he seems inclined to assume that in phosphorus-poisoning alteration in the autolytic enzymes is an important factor in the liver degeneration. It would seem much more probable that phosphorus is a poison that kills cells and does not destroy their autolytic enzymes, hence favoring autolysis. The liver degeneration following chloroform poisoning may, perhaps, be explained in a similar way, the cells behaving exactly as bacteria would do under the same conditions. Taylor³ has analyzed several livers in degenerative conditions for amino-acids and found them only in one liver, which showed necrosis probably due to chloroform poisoning, and which was from a case clinically resembling acute yellow atrophy. Here he obtained 4 gm. of leucin, 2.2 gm. of tyrosin, and 2.3 gm. of arginin nitrate. Waldvogel and Tintemann,⁴ in phosphorus livers, found an increase in protagon, jecorin, fatty acids, cholesterolin, and neutral fat, while lecithin was decreased. Wake-man⁵ found arginin, histidin, and lysin decreased in phospho-

¹ Zeit. klin. Med., 1904 (55), 508.

² Zeit. f. physiol. Chem., 1900 (30), 174.

³ Univ. of Calif. Public. (pathol.), 1904 (1), 43.

⁴ Cent. f. Path., 1904 (15), 97.

⁵ Berl. klin. Woch., 1904 (41), 1067.

rus livers in proportion to the total nitrogen, indicating that the proteid-splitting enzyme in this condition either picks out certain varieties of proteids first, or removes the nitrogen-rich constituents most rapidly.

It is probable that many poisons may destroy the liver cells to such an extent that they cannot maintain their normal chemical equilibrium, without, at the same time, destroying the autolytic enzymes. When this occurs, the liver undergoes autolysis, and we get marked degenerative changes with appearance of amino-acids in the blood and urine, reduction in coagulability of the blood and numerous hemorrhages, giving a picture both clinically and anatomically more or less like that of typical acute yellow atrophy. Chloroform is a poison that stops cell activities without destroying the proteolytic enzymes, hence the cells undergo autolysis, and, as a result, we have many cases of what appears to be acute yellow atrophy following chloroform anesthesia. (See "Acute Yellow Atrophy," Chap. xviii.) Probably the liver changes in puerperal eclampsia, and in streptococcus and other septicemias are of a similar nature.¹

Postmortem changes are undoubtedly due to two factors, bacterial action and autolysis. In tissues kept at a low enough temperature to exclude bacterial action, but not so low as to absolutely stop enzyme action, there occurs a slow autolysis; this constitutes the "ripening" process of meat. Fish flesh may also ripen when made sterile in saturated salt solutions, as Schmidt-Nielsen² has shown occurs with salted herrings, oxy-acids and xanthin bases being prominent among the products. The softening of muscles in rigor mortis is probably also an autolytic manifestation, as muscles contain proteases acting best in acid medium, and the muscle is known to become increasingly acid after circulation ceases within it. The short duration of rigor mortis when the body is kept warm, and its early disappearance when death has been preceded by muscular exhaustion (which increases the acidity), agree with this view. The early postmortem softening of many organs in pathological conditions is also probably an autolytic manifestation. Flexner³ has called attention to this in relation to the softening of the parenchymatous organs in acute infectious diseases, such as typhoid and septicemia. Schumm⁴ noted great autolytic activity in a swollen spleen from a case of perityphlitis.

Histological changes are produced by autolysis in the organs

¹ Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

² Hofmeister's Beiträge, 1903 (3), 267.

³ Loc. cit.

⁴ Loc. cit., *infra*.

after death that are, as might be expected, much like those seen in necrotic areas.¹ At first the changes resemble those of parenchymatous degeneration (cloudy swelling), and often there is an apparent increase in fat, which is probably due to liberation of masked fat through the destruction of the proteid.² Nuclear staining is lost (karyolysis), and eventually even cell forms become indistinguishable, but this does not ordinarily become complete in autolysis without bacterial complication.

Still-born children that have been carried for some time after death usually show considerable disintegration of the viscera, especially the liver. This is undoubtedly due to autolysis, which Schlesinger³ has shown can begin before birth if the fetus dies in utero.

Autolysis in Relation to Infection.—According to Conradi⁴ the substances produced in tissue autolysis have a decided inhibiting effect upon bacteria, which apparently depends upon the antiseptic properties of the aromatic derivatives that are split out of the proteid molecule in autolysis. This action is manifested not only *in vitro*, but the autolytic products will also render harmless lethal doses of certain bacteria if they are injected simultaneously with the bacteria into an animal. It may well be questioned, however, whether enough of these substances ever accumulates in infected tissues during *intra vitam* autolysis to have much effect upon the infecting bacteria; yet this property may possibly explain the sterilization of old pus collections and similar infected accumulations within the body. The bacteria themselves also produce autolytic products that are powerfully bactericidal. (See "Bacteria," Chap. iv).

Blum⁵ found that the autolytic products of lymph-glands neutralized tetanus toxin, but were inactive against diphtheria toxin and cobra venom. Products from other autolyzed organs and from fresh lymph-glands were without influence on the tetanus toxin. The antitoxic principles of the autolytic product were destroyed by heating, weakened by acids and alkalies, and in other respects showed properties strikingly like those of true antitoxin. It is quite possible that bacterial toxins may be destroyed by autolytic enzymes, for Baldwin and Levene⁶ have shown that trypsin, pepsin, and papain destroy tetanus and diph-

¹ More fully discussed by Wells, Jour. Med. Research, 1906 (15), 149.

² Siegert (Hofmeister's Beitr., 1901 (1), 114) found no actual increase in fats and fatty acids in autolysis even when an increase was apparent histologically, although ether-soluble materials of other nature than fat may be increased.

³ Hofmeister's Beitr., 1903 (4), 87.

⁴ Hofmeister's Beitr., 1901 (1), 193.

⁵ Hofmeister's Beitr., 1904 (5), 142.

⁶ Jour. Med. Research, 1901 (6), 120.

theria toxin, while tuberculin is destroyed by trypsin, but not readily by pepsin, possibly because it is of a nucleoproteid nature.

On the other hand, there are many pathogenic bacteria which do not secrete their toxic materials, but store them up within the cell body, *e. g.*, typhoid, cholera, and, indeed, the majority of pathogenic forms. These *endotoxins* are probably liberated from the bacteria only through digestion of their cells, either by their own autolytic enzymes, or by the enzymes of the infected tissues and leucocytes.

Leukemia.—The abundant elimination of uric acid and other purin bodies in the urine in leukemia testifies to the great amount of destruction of nucleoproteid that is going on during the disease, and this is probably derived from the autolysis of leucocytes. Schumm¹ has studied the autolytic changes in a spleen from a case of acute leukemia (variety not stated) with the following results: The leukemic spleen immediately after death contains much proteose, and this soon disappears, while leucin, tyrosin, lysin, and ammonia appear, and the proteid constituents disappear. In a later communication² he reported the findings in the autolyzed spleens of two cases of splenomyelogenous leukemia. He detected among the products guanin, xanthin, hypoxanthin, histidin, lysin, alanin, leucin, tyrosin, thymine, paralactic acid, and ammonia; adenin and arginin were not found. Autolysis of the leukemic bone-marrow produced tyrosin, leucin, and tryptophan. In fresh leukemic blood he found much albumose as well as an enzyme digesting casein in alkaline medium. Autolysis of the leukemic spleen is more complete than that of the normal spleen. v. Jaksch,³ Erben,⁴ and others have noted the occurrence of peptones and albumoses in leukemic blood, particularly if removed postmortem. The improvement in leukemia that follows x-ray treatment is associated with an increased nitrogen elimination, probably due to autolysis of disintegrating cells.⁵ (See also "Leukemia," Chap. xi.)

Tumors.—Probably because of the great amount of necrosis that is constantly going on in all malignant growths, with subsequent digestion of the dead cells, autolytic products are present in them in very considerable amounts. This was first

¹ Hofmeister's Beitr., 1903 (3), 576.

² *Ibid.*, 1905 (7), 175.

³ Zeit. f. physiol. Chem., 1892 (16), 243.

⁴ Zeit. f. klin. Med., 1900 (40), 282; Zeit. f. Heilkunde, 1903 (24), 70; Hofmeister's Beitr., 1904 (5), 461.

⁵ Musser and Edsall, Univ. Penn. Med. Bull., 1905 (18), 174.

demonstrated by Petry,¹ who found that carcinomata of the breast contained much of their nitrogen in compounds not coagulated by heat, while in the normal gland practically all is coagulable. He also demonstrated an autolytic property in tumor tissue, showing that tumor cells do not differ in this respect from normal cells.

Neuberg² found that while, according to other observers, most enzymes, as well as bacteria, are very susceptible to the action of radium rays, the autolytic enzymes of cancer cells are an exception, for cancer tissue exposed to radium undergoes autolysis much faster than cancer tissue not exposed to radium. He attributes the effects of radium on cancer to its deleterious effects on the oxidizing and other enzymes of the cells, destroying their activities, which results in destruction of the cells by the autolytic enzymes.³ A cancer of the stomach was found to contain autolytic enzymes capable of digesting lung tissue (pepsin was excluded) and autolyzed cancers yielded much pentose. Blumenthal and Wolf⁴ believe that tumor tissues have particularly active autolytic enzymes, since liver tissue added to tumor tissue underwent autolysis much more rapidly than normal. Beebe⁵ found products of autolysis constantly present in several tumors; namely, a carcinoma of the broad ligament, a hypernephroma, an angiosarcoma, and a round-cell sarcoma.

Micheli and Donati⁶ attribute the hemolytic properties possessed by extracts of malignant tumors to the products of autolysis that are present, which Petry has also demonstrated to produce hemolysis. Emerson⁷ attributes the disappearance of HCl from the gastric juice in carcinoma of the stomach to neutralization by basic products of autolysis, a hypothesis that may well be questioned. (See also "Tumors," Chap. xvii.)

Various other intracellular enzymes have been described, which for the most part have as yet no significance in pathology. An exception is *fibrin ferment*, which will be considered fully in discussing thrombosis. Ferments coagulating milk seem to be widely spread in the tissues.

¹ Zeit. f. physiol. Chem., 1899 (27), 398; Hofmeister's Beitr., 1902 (2), 94.

² Zeit. f. Krebsforschung, 1904 (2), 171; Berlin. klin. Woch., 1904 (41), 1081; *Ibid.*, 1905 (42), 118.

³ Wohlgemuth, Berl. klin. Woch., 1904 (41), 704, found that autolysis in tuberculous lung tissue was three or four times more rapid when exposed to radium rays. Heile (Arch klin. Chir., 1905 (77), 107) looks upon the favorable effects of x-rays as partly produced by their liberation of autolytic enzymes from the leucocytes.

⁴ Med. Klinik, 1905 (1), No. 7.

⁵ Amer. Jour. Physiol., 1904 (11), 139.

⁶ Riforma med., 1903 (19), 1037.

⁷ Deut. Arch. klin. Med., 1902 (72), 415.

The precipitation of plastein from proteose solution by organ extracts (Nürnberg) may be either the effect of a coagulating ferment or due to reverse action of the proteases. Ferments splitting specifically maltose, starch, and nucleoproteids have been described, and the glycogenic ferment is probably nearly universally present. Other enzymes decomposing amino-acids into ammonium compounds may also exist. The enzymes acting specifically upon the nucleic acids and the purin bodies have already been discussed.

CHAPTER IV

THE CHEMISTRY OF BACTERIA AND THEIR PRODUCTS

STRUCTURE AND PHYSICAL PROPERTIES¹

IN structure, as in nearly all other respects, bacterial cells stand intermediate between the cells of ordinary plant and animal tissues. Their cell wall seems to be generally more highly developed than that of animal cells, and less so than the wall of most plant cells. In composition, however, the wall is more closely related to animal than to vegetable tissues. The much-vexed question as to the existence or non-existence of a nucleus seems to be best answered by Zettnow, who considers that the portion of the bacterial cell usually made evident by ordinary staining methods consists of a mixture of nuclear substance (*chromatin*) with non-chromatic substance (*entoplasm*); the outer membrane, which requires special methods for its satisfactory demonstration, consists of a modified cytoplasm (*ectoplasm*). Some bacteria consist chiefly of chromatin (*e. g., vibrios*), but the proportion of the different elements varies greatly, not only in different varieties, but also in the same variety under different conditions. The fact that the chromatin is not aggregated into the usual nuclear form may be ascribed to the low stage of development reached by bacteria in the scale of evolution; or, as Vejdovsky has suggested, to the extremely rapid rate of cell division in the bacteria which prevents the chromatin from appearing in the resting stage which a nucleus constitutes. Finer structures within the bacterial cell have as yet been only imperfectly discerned.

The thickness of the ectoplasm varies greatly even in the same species, being generally greatest in older cultures. In some forms the ectoplasm may constitute one-half of the total mass of the cells. The capsule seems to arise through a swelling of the ectoplasm, and is probably present in at least a rudimentary stage in all bacteria (Migula).

¹ In this chapter references will not generally be given that can be found by consulting Kolle and Wassermann's Handbuch. A general consideration of the Biology of the Bacteria, including references to the effects of light, heat, osmotic pressure, etc., is given by Müller, *Ergeb. der Physiol.*, 1904 (4), 138.

Plasmolysis and Plasmoptysis.—Under conditions of altered osmotic pressure the bacterial cell behaves quite similarly to the plant cell.¹ If placed suddenly in a solution of higher osmotic pressure than the one in which it has been, the cell contents shrink away from the cell wall (*plasmolysis*) indicating that there exists a semipermeable membrane through which water passes more rapidly than salts. If the change in osmotic pressure is gradual, the bacteria accommodate themselves to it by the slow diffusion of the salts through the cell membrane, indicating that it is not absolutely semipermeable. Different bacteria behave differently, some bacteria not being plasmolyzed by solutions that plasmolyze others. As a rule, old bacteria plasmolyze more rapidly than young, and in some varieties there seems to be a spontaneous plasmolysis, to which has been attributed the irregular staining of diphtheria and tubercle bacilli, the polar staining of plague bacilli, etc. Plasmolysis occurs only in living bacilli, but does not necessarily cause death.

When bacteria pass from solutions of higher osmotic concentration into solutions of lower concentration, the phenomenon of *plasmoptysis* is produced. The cell contents swell until the cell wall gives way at some point, and then exude as glistening drops, which may become detached from the wall and escape free into the fluid. Plasmoptysis is shown best by bacteria that have been grown on salt-rich media before being placed in the salt-free fluid. Not all varieties of bacteria can be made to undergo this change, depending probably upon a greater permeability of their cell membranes for salts. The exposure of the naked cell contents to the hypotonic fluid outside the cells makes plasmoptysis more serious for bacterial life than plasmolysis, but how often either process plays a part in the resistance of infected animals against bacteria is unknown.

Chemotaxis.—Just as with unicellular animal organisms, bacteria respond to chemotactic influences, in general being attracted by substances favorable for food, such as peptone, dilute potassium salts, etc., and being repelled by harmful substances, such as strong acids and alkalies. Attempts have been made to separate different organisms in mixed cultures by means of their response to chemotaxis, but without striking success. It is possible that chemotaxis may play a part in the localization of bacteria from the blood stream in favorable localities, just as leucocytes are attracted to points of injury, but this has not

¹ Literature, see Gotschlich, Kolle and Wassermann's *Handbuch*, 1903, vol. 1, p. 62.

been demonstrated. (The chemotactic influence of bacteria upon leucocytes is discussed in Chapter x.)

CHEMICAL COMPOSITION

This varies greatly, not only between different species, but even in the same species grown on different media; in this respect bacteria are much more modified by their environment than are higher organisms. Grown on a salt-rich medium they yield much ash; grown on a peptone-rich medium they contain much proteid; grown on a fat-rich medium they contain much material soluble in ether. Cholera vibrios grown on a bouillon medium contained 69.25 per cent. of proteid, and 25.87 per cent. of ash, whereas the same organism grown on Uschinsky's medium, which contains no proteids but only various simple chemical compounds,¹ contained but 35.75 per cent. of proteid and 13.7 per cent. of ash (Cramer). Even in the same medium two different strains of the same organism may show equally great differences: Two strains of cholera vibrios grown on the same medium showed respectively 65.63 per cent. and 34.37 per cent. of proteid. It is evident, therefore, that quantitative analyses of bacteria show nothing as to their nature, and on account of the extreme limits of their variation are practically valueless.

Qualitatively the variations are not so great—all bacteria contain proteids, lipid substances, and salts, of which phosphates are most prominent in the ash. The older analyses of bacterial constituents are of little value. Recent studies prove that the chief constituent of the cell contents is a true nucleoproteid (Iwanoff²) containing some sulphur and iron; probably many of the "pyogenetic proteids," "bacterial toxalbumins," "bacterial caseins" of earlier investigators are true nucleoproteids. In a water bacillus Nishimura found xanthin, guanin, and adenin, indicating the presence of nucleoproteid; others have found that bacterial nucleoproteids split off pentoses, as do the nucleoproteids of higher cells. Mary Leach³ found evidence that the colon bacillus is largely made up of nuclein or glyconucleoproteids, but contains no cellulose. Other proteids, namely, globulins and nucleo-albumins, have also been described

¹Uschinsky's medium is: Water, 1000 parts; glycerin, 30-40; sodium chloride, 5-7; calcium chloride, 0.1; magnesium sulphate, 0.2-0.4; di-potassium-phosphate, 0.2-0.25; ammonium lactate, 6-7; sodium asparaginate, 3-4 parts.

²Hofmeister's Beit., 1902 (1), 524.

³Jour. Biol. Chem., 1906 (1), 463. Full bibliography on Chemistry of Bacteria.

as constituents of the bacterial plasma. The slimy material produced in cultures by some varieties of bacteria is, at least for certain forms, a body closely related to or identical with true mucin.¹ Heim² considers that anthrax bacilli also produce mucin.

Bacterial Carbohydrates.—Likewise the earlier descriptions of *cellulose* or *hemicellulose* in the cell membrane of bacteria are undoubtedly incorrect. Numerous investigations have shown that the insoluble bacterial cell wall consists chiefly of *chitin*, which on being split with acids yields 80 to 90 per cent. of the nitrogenous carbohydrate, *glucosamin*. The distinction is a very important one, since cellulose is a typically vegetable product, while chitin is equally typically animal in origin, being found chiefly in the shells of lobsters and crabs, the wings and coverings of flies, beetles, etc. Chitin seems to be an amino-derivative of a carbohydrate, a polymeric form of some simpler compound, just as cellulose is a polymer of a simpler carbohydrate.

Other carbohydrates seem to be scanty in the bacterial cell. Cramer could find no glucose in any variety, although there are some bacteria that contain material reacting like starch with iodine. Levene,³ however, found in *B. tuberculosis* a substance with the properties of glycogen.

Bacterial Fats.—By staining methods, fats have been recognized in many species, and by extraction with fat solvents lecithin, cholesterolin, simple fats, and specific bacterial fats have been isolated; this is particularly true of *B. tuberculosis*, which owes its characteristic staining properties to the specific fat-like bodies which make up a large proportion of its entire mass.⁴ Numerous studies of these fats of *B. tuberculosis* have been made⁵ and by using different extractives, from 20 to 40 per cent. of the entire weight of the bacilli has been found soluble in fat solvents. Kresling found that the substance soluble in chloroform had the following composition:

Free fatty acid	14.38 per cent.
Neutral fats and fatty acid esters	77.25 " "
Alcohols obtained from fatty acid esters	39.10 " "
Lecithin	0.16 " "
Substances soluble in water	0.73 " "

¹ Rettger, Jour. Med. Research, 1903 (10), 101.

² Münch. med. Woch., 1904 (51), 426.

³ Jour. Med. Research, 1901 (6), 135.

⁴ See Camus and Pagniez, Compt. Rend. Soc. Biol., 1905 (59), 701.

⁵ For literature see Bulloch and Macleod, Jour. of Hygiene, 1904 (4), 1.

Bulloch and Macleod found that ethereal extracts did not contain the acid-fast substances which they consider to be a wax-like alcohol, soluble in hot, but insoluble in cold absolute alcohol or in ether. The simple fats seem to be formed by *oleic*, *isoctenic*, and *myristinic* acids, and there is some *lauric* acid in the form of a soap. Cholesterin is probably present, and there are also lipochromes giving the cultures their color.

By staining with sudan III, Sata¹ demonstrated fats, not only in the acid-fast bacilli, but also in anthrax, *Staphylococcus aureus*, *B. mucosus*, and actinomyces; but not in diphtheria, pseudo-diphtheria, plague, cholera, and chicken cholera bacilli, or in members of the colon group.² Only a few bacteria form fat on agar free from glycerin, but potato is a favorable medium.

Spores differ from their parent bacteria in containing a much greater proportion of the solid constituents and less water. In molds Drymont found that the spores contained over 60 per cent. of dry substance, and almost all the water was so held as to resist drying by temperatures below boiling; the dry substance is very rich in proteid and poor in salts. The wall of the spore consists of a "cellulose-like" substance (probably chitinous) and a very hygroscopic extractive matter. The great resistance of spores to drying and to heat can be readily understood in view of these facts. Flagella also seem to be composed of a relatively condensed proteid.

Staining Reactions.—The staining reactions of bacterial cells are much as if the bacteria consisted entirely of chromatin, so that at one time the theory prevailed that bacteria consisted merely of a nucleus and a cell wall, without any true cytoplasm. The demonstration of abundant nucleoproteid in the contents of bacterial cells explains their staining affinity for basic anilin dyes. Owing to some unknown differences in composition, not all bacteria are stained equally well by the same basic dyes. Although the staining of bacteria depends upon a chemical reaction between the nucleoproteids and the basic dye, yet the combination is not usually a firm one, being readily broken by weak acids in most cases. That the decolorization of bacteria depends upon dissociation of the dye-proteid compound is shown by the fact that absolutely water-free alcohol will not decolorize dry bacteria, nor do water-free alcoholic solutions of dyes stain dehydrated bacteria.

¹ Cent. f. allg. Path., 1900 (11), 97.

² Auclair (Arch. Méd. Exper., 1903 (15), 725) contends that the ether and chloroform extracts of many pathogenic bacteria contain important toxic substances. Holmes (Guy's Hosp. Reports, 1905 (59), 155) states that injection of fatty acids from tubercle bacilli into rabbits causes a lymphocytosis.

Gram's method of staining depends on the formation of an iodine-pararosanilin-proteid compound which is not easily dissociated by water in the case of bacteria that stain by this method, and which is readily dissociated and dissolved out in the case of bacteria that do not retain the stain. Only pararosanilin dyes (gentian violet, methyl violet, victoria blue) form such combinations, the rosanilin dyes (fuchsin, methylene-blue) not being suitable.

The acid-fast bacilli (leprosy, tubercle, and allied forms) owe their characteristic resistance to both staining and destaining processes, to their high fat content, which modifies greatly the penetration by stains and reagents. It is said that organisms not ordinarily acid-fast may be made so by increasing their fat contents by growing them on fat-rich media. According to Bulloch and Macleod¹ the acid-fastness of the tubercle bacillus depends not on the ordinary ether-soluble fats, but on a high molecular alcohol of undetermined composition, soluble in boiling absolute alcohol.

BACTERIAL ENZYMES

The metabolic processes of bacteria seem to be closely dependent upon enzyme action, just as with higher cells.² Liquefaction of gelatin is a familiar example of the enzyme action of bacteria; and since the filtered cultures of liquefactive bacteria are also capable of digesting gelatin, the enzymes are evidently excreted from the cells. Dead bacteria, killed by thymol or by other antiseptics that do not destroy proteolytic enzymes, will also digest gelatin. Numerous investigations have established the wide-spread occurrence of many soluble enzymes both in bacteria

¹ *Loc. cit.*

² One must distinguish between "enzymes" and "ferments," although since most of the characteristic fermentative actions of yeast and other cells have been found to be produced by intracellular enzymes, the distinction is not always easy to make. Gotschlich (Kolle and Wassermann's Handbuch, vol. i, p. 104) would distinguish them as follows: "Fermentation is a direct function of the living protoplasm, and serves as its source of energy." "Enzyme action is not directly dependent on the living protoplasm, and does not serve the organism as a source of energy." Exception can readily be taken to these definitions, however, for the latest indications are that nearly all of the separate processes that go to make up the process of fermentation are enzyme processes. Fermentation may, therefore, be looked upon as the action of living organisms, being the sum of the action of the enzymes of the organisms together with certain other chemical processes not brought about by enzymes. In general, the distinction is made chiefly on the ground that we can stop fermentative processes by means of certain antiseptics that kill the causative organisms, but which do not greatly impair the enzymes. Even this distinction is more quantitative than qualitative, for very dilute solutions of enzymes are nearly as susceptible to antiseptics as are bacteria (Kaufmann, *Zeit. physiol. Chem.*, 1903 (39), 434).

and in their secretions, indicating that bacterial cells are as dependent on enzymes for the production of their metabolic activities as are higher types of cells, and that these enzymes are not only present as intracellular constituents, but that they also escape from the cells.

The diffusion method of Wijsman, or, as it is more frequently called, *auxanographic* method of Beijerinck, offers a relatively simple means of detecting the presence of extracellular bacterial enzymes. Eijkman¹ in particular has used this method, which consists of mixing agar with milk, or starch, or whatever material is to serve as the indicator of the enzyme action; the agar is then inoculated with bacteria and plated (or else the bacteria are inoculated as a streak on the surface of the agar). About each colony there will appear a zone of clearing in the medium, if it produces enzymes digesting the admixed substance. By this means Eijkman found that all bacteria that produce enzymes digesting gelatin also digest casein, and those that do not digest gelatin are equally without effect on casein; therefore, it is probably the same enzyme that digests both. As the hemolytic action of bacteria is not constantly related to their gelatin-dissolving property, the hemolysis probably is produced, at least in some cases, by other means than the proteolytic enzymes. A few pathogenic bacteria (anthrax, cholera) digest starch, and *B. pyocyaneus*, *Staphylococcus pyogenes aureus*, and *B. prodigiosus* all produce fat-splitting enzymes. *B. pyocyaneus*, he found, digested elastic tissue readily,² as also did a bacillus resembling *B. subtilis* obtained from the tissue of a gangrenous lung. The following table by Buxton³ gives an idea of the distribution of enzymes in bacterial secretions as determined by the auxanographic method:

ENZYMES HYDRATING CARBOHYDRATES

	Amylase.	Maltase.	Invertase.	Lactase.	Inulase.
1. Anthrax	+	+	—	—	—
2. Cholera	+	+	—	—	—
3. Coli communis	—	+	—	+	—
4. Typhoid	—	+	—	—	—
5. Diphtheria	—	—	—	—	—
6. Staph. pyogenes aureus	—	+	—	—	—
7. Lactis aërogenes	—	+	+	+	—
8. Pyocyaneus	—	—	—	—	—
9. Violaceus	—	—	—	—	—
10. Mycoides	+	+	—	—	—
11. Prodigiosus	—	—	—	—	—
12. Saccharomyces niger	—	+	+	—	—
13. Saccharomyces neoformans	—	+	+	—	—
14. Aspergillus niger	+	+	+	—	+
15. Aspergillus oryzae	+	—	+	—	—

¹ Cent. f. Bakt., 1901 (29), 841.

² Cent. f. Bakt., 1903 (35), 1.

³ American Med., 1903 (6), 137.

PROTEOLYTIC ENZYMES, DIGESTING

	Milk.		Gela- tin.	Serum.	Egg- albu- men.	Fibrin.	Red blood- corpus- cles.
	Coagul.	Diges- tion.					
1. Anthrax	+	+	+	—	+	+	+
2. Cholera	+	+	+	+	+	+	+
3. Coli communis	—	—	—	—	—	—	—
4. Staph. pyogenes aureus	+	+	+	—	—	—	+
5. Streptococcus pyog- enes	—	—	—	—	—	—	+
6. Pyocyanus	+	+	+	+	+	—	?
7. Violaceus	—	—	+	—	—	—	+
8. Mycoides	+	+	+	—	+	—	+
9. Prodigiosus	+	+	+	+	+	+	+
10. Aspergillus niger . .	+	+	+	—	—	—	+
11. Aspergillus oryzae . .	+	+	+	+	+	—	—

Rennin is produced by many bacteria, as is shown by their coagulating milk, independent of any acid reaction.¹

An interesting observation made by Schmailowitsch² is that the amount and nature of enzymes produced by bacteria is modified by the amount and nature of their food. When they receive no food, they secrete no enzymes; when grown on proteid-rich media they produce much proteolytic enzyme; grown on a carbohydrate medium they produce chiefly amylolytic enzymes. This observation recalls Pawlow's demonstration of the similar influence of the quality of food upon the proportion of the various digestive enzymes contained in the pancreatic juice; under proteid diet the trypsin is in excess; under starch diet the amyllopsin is in excess, etc. Abbott and Gildersleeve³ have corroborated this statement, finding that bacteria grown on gelatin produce much more active gelatin-dissolving enzyme than do bacteria grown on bouillon. This phenomenon they would explain on the basis of Welch's hypothesis that bacteria react to chemical substances by producing antagonistic substances, just as higher organisms do under similar conditions. It is probably closely related to the difference of composition observed in bacteria grown on different media (*vide supra*).

In general, bacterial proteolytic enzymes resemble trypsin more closely than they do pepsin, acting best in an alkaline medium; but the enzymes extracted from bacterial cultures are very feeble as compared with pancreatic trypsin. Abbott and Gildersleeve found that the gelatin-dissolving enzyme of bacteria resists a temperature of 100° C. for as long as fifteen to

¹ Contradicted by DeWaele, Cent. f. Bakt., 1905 (39), 353.

² Wratschebnaja Gazetta, 1902, p. 52.

³ Jour. Med. Research, 1903 (10), 42.

thirty minutes (disagreeing with Fermi). Schmailowitsch¹ states that some bacteria produce an enzyme acting in acid medium upon gelatin but not upon albumin, and this enzyme carries the digestion only as far as the gelatin-peptone stage, whereas the enzymes acting in an alkaline medium carry the splitting through to leucin, tyrosin, etc. Plenge² suggests that there is a special enzyme digesting nucleoproteids. The bacterial amylolytic enzyme acts like ptyalin.

Cacace³ investigated the splitting products of gelatin and coagulated blood when digested by *B. anthracis*, *Staph. pyogenes aureus*, and *Sarcina aurantiaca*, and found that proteoses and peptone are produced, which disappear in the later stages of digestion. Rettger⁴ found leucin, tyrosin, tryptophan, as well as phenols, skatol, indol, aromatic oxy-acids, and mercaptan, among the products of bacterial decomposition of egg-albumen and meat; proteoses and peptones appear in the early stages, but later disappear, as also eventually do the leucin, tyrosin, etc. Cholin has also been found in the products of autolysis.⁵ Mavrojiannis⁶ found that some bacteria digest gelatin only as far as the gelatose stage (which is determined by its being hardened by formalin), while others carry the digestion to peptones and non-proteid substances which cannot be hardened by formalin.

The digestive power of the filtrates of cultures and of killed bacteria is far less than that of the living bacteria (Knapp⁷). Streptococci digest proteids of exudates feebly, staphylococci more rapidly, and colon bacilli are still more active. He could find no relation between the proteolytic power of the bacteria and the severity of the infection from which they came.

Immunity against bacterial enzymes may be secured as it is against other enzymes. Abbott and Gildersleeve⁸ found that by injections into animals of proteolytic bacterial filtrates which were only slightly toxic, the serum of the animals acquired a slight but specific increase in resistance to the proteolytic enzymes of the filtrates. Normal serum contains a certain amount of enzyme-resisting substance. Other observers have found that immunization against living or dead bacteria leads to the production of substances antagonistic to their

¹ Abst. in Biochem. Centr., 1903 (1), 230; see also DeWaele, Cent. f. Bakt., 1905 (39), 353.

² Zeit. f. physiol. Chem., 1903 (39), 190.

³ Cent. f. Bakt., 1901 (30), 244.

⁴ Amer. Jour. of Physiol., 1903 (8), 284.

⁵ Kutscher and Lohmann, Zeit. physiol. Chem., 1903 (39), 313.

⁶ Zeit. Hygien. u. Infektionskr., 1903 (45), 108.

⁷ Zeit. f. Heilk. (Chir. Abt.) 1902 (23), 236.

⁸ Loc. cit.

enzymes, but the degree of resistance acquired is never great. v. Dungern¹ found that the serum of animals infected with various bacteria prevented digestion of gelatin by the enzymes obtained from cultures of the same species of bacteria. He applied this fact to the diagnosis of infectious conditions, finding that the serum of a patient with osteomyelitis was over twenty times as strongly inhibitory to staphylococcus enzymes as was serum of normal persons. The reaction is specific, cholera vibrio enzymes not being inhibited to any corresponding degree.

Autolysis of Bacteria.—Autolysis occurs also in bacteria, their proteolytic enzymes digesting the cell substance whenever the organisms are killed by agents (chloroform, toluol, etc.) that do not destroy these enzymes. Even the absence of food leads to the same result, presumably because the normally existing autolytic processes are not counteracted by synthesis of new proteid material; hence, autolysis occurs when bacteria are placed in salt solution or distilled water. Although it had been known for many years that yeast cells digest one another when there is nothing else for them to live upon, the first definite study of bacterial autolysis seems to have been made by Levy and Pfersdorff² and Conradi.³ The former digested anthrax bacilli (in whose bodies are contained rennin, lipase, and protease) under toluol for several weeks, and obtained a slightly toxic product. Conradi permitted dysentery bacilli and typhoid bacilli to digest themselves in normal salt solution for twenty-four to forty-eight hours at 37° C., and obtained in this way the soluble, highly poisonous endotoxins of the bacteria, which are liberated by the destruction of the bacterial structure by the autolytic enzymes. Longer autolysis results in the destruction of the endotoxins themselves by the enzymes. Rettger⁴ found among the autolytic products of bacteria, leucin, tyrosin, basic substances, and phosphoric acid. Under favorable conditions complete autolysis can occur in two to ten days.

Brieger and Mayer⁵ found that at room temperature (15° C.) practically no autolysis occurs with typhoid bacilli in distilled water, and the soluble products thus obtained are quite non-toxic, although if injected into animals they give rise to the production of agglutinins and bacteriolysins. Bertarelli⁶ has

¹ Münch. med. Woch., 1898 (45), 1040.

² Deut. med. Woch., 1902 (28), 879.

³ *Ibid.*, 1903 (29), 26.

⁴ Jour. Med. Research, 1904 (13), 79.

⁵ Deut. med. Woch., 1904 (30), 980.

⁶ Cent. f. Bakt., 1905 (38), 584.

used the products of autolysis of cholera vibrios successfully in the production of immunity, and states that the products of autolysis consist largely of nucleins.

It is probable that in every culture bacteria are constantly being destroyed, either by their own enzymes or by the proteolytic enzymes of the other bacteria. Some bacteria are much more rapidly autolyzed than others, cholera vibrios, colon, typhoid, and dysentery bacilli being rapidly digested, while tubercle bacilli are very little and slowly autolyzed. Conradi¹ who has shown that certain products of autolysis of tissues are bactericidal, believes that also in cultures powerfully bactericidal substances are produced through autolysis of the bacteria. This, he thinks, accounts for the decrease in numbers of living bacteria that always sets in after a short period of growth on artificial media; for example, the bacteria in a culture of cholera vibrios increase in number for about twelve hours, and then their number steadily decreases. When cultures that have ceased to grow are placed in a diffusion membrane, so that the autolytic products can escape, growth promptly begins again.² It has been found by Turró³ that extracts from various tissues containing autolytic enzymes can digest bacterial cells.⁴ It is very possible that the endotoxins contained within such pathogenic bacteria as typhoid and cholera are liberated through digestion of the bacteria, either by autolysis or by the enzymes of the leucocytes and tissues of the organism that they have infected. These, and a number of other bacteria, produce no soluble toxins that diffuse from the cells, as do diphtheria and tetanus toxin, and it is difficult to explain the toxic effects these bacteria produce without assuming that their intracellular toxins are liberated in some such way. It is also quite probable that the enzymes found in filtrates from bacterial cultures are liberated from the bacterial cells only when these have been autolyzed.⁵ With the possible exception just mentioned, there is little evidence that the bacterial enzymes play any important rôle in infectious diseases. They may be a slight factor in the diges-

¹ Münch. med. Wochenschr., 1905 (52), 1761.

² The conclusions of Conradi are contested by Manteufel, Berl. klin. Woch., 1906 (43), 313.

³ Cent. f. Bakt., 1902 (32), 105.

⁴ Sigwart (Arb. a. d. Path. Inst. Tübingen, 1902 (3), 277) found that trypsin and pepsin (without acid) do not injure living anthrax bacilli.

⁵ Emmerich and Loew (Zeitschr. f. Hyg., 1899 (31), 1), having found that *pyocyanase* is capable of destroying and digesting other bacteria than *pyocyanus*, suggested that it might be a potent factor in producing artificial immunity. Their rather remarkable hypotheses have been much contested, and are of questionable value. (See Petrie, Jour. of Pathol. and Bacteriol., 1903 (8), 200; also, Rettger (Jour. Infectious Diseases, 1905 (2), 562).

tion of tissue and exudates in suppuration, but as compared with the leucocytic enzymes their influence is probably minute; beyond this they have no apparent influence upon their host, and are chiefly concerned in the metabolism of the bacteria. The proteoses and peptones produced by bacterial action do not seem to be any more toxic than those produced by pepsin and trypsin.

POISONOUS BACTERIAL PRODUCTS

Almost without exception all the harm that bacteria do is brought about by means of the chemical substances produced in one way or another by their metabolic processes. Animal parasites may do harm mechanically, but with the possible exception of the effects of capillary emboli (especially with anthrax), bacteria produce all their effects through chemical means. The poisonous chemical substances produced by bacteria may be grouped into four classes:

I. Products of the decomposition of the media upon which the bacteria are growing; among these the best known are the *ptomaïns*.

II. Soluble poisons manufactured by the bacteria, and secreted from the cell into its surrounding media—the true *toxins*.

III. Poisons manufactured by the bacteria which do not escape from the normal cell, but which are as specific in their poisonous properties as the true toxins; because of their intracellular situation they are called *endotoxins*.

IV. Poisonous proteid constituents of the bacterial cell, which form part of the cell protoplasm, but which are not soluble and the poisonous effects of which are not specific and not usually responsible for the disease; these are called *bacterial proteids*.

PTOMAÏNS

Ptomaïns, the soluble basic nitrogenous substances that are found in the medium in which bacteria have been growing, were the first bacterial products that were recognized, and for some time it was believed that it was through the production of such alkaloid-like substances that bacteria caused disease, just as poisonous plants owe their effects to poisonous alkaloids. It was soon found, however, that the ptomaïns that could be isolated from cultures of pathogenic bacteria were insufficient by themselves to cause the poisonous effects that such cultures produced when injected into animals. The isolated ptomaïns were not only far less poisonous than the original culture, but furthermore they did not produce the symptoms and anatomical

changes characteristic of the diseases that the pathogenic organism caused. Furthermore, the majority of ptomaines are not very poisonous, and highly poisonous ptomaines may be produced by non-pathogenic bacteria. As a result, the work on ptomaines, which twenty years ago occupied many laboratories and promised to reveal the entire chemistry of bacterial intoxication, has now been almost completely dropped. The interest in ptomaines is by no means entirely historical, however, for poisonous ptomaines at times do enter the body and cause illness, sometimes even death. The close chemical resemblance to vegetable alkaloids of some of the ptomaines that may arise in decomposing corpses, makes them of great importance to chemists searching for the cause of death in cases of supposed poisoning. Therefore the most essential features of the ptomaines and their chief known relations to intoxications will be briefly discussed, referring the reader for a full consideration to Vaughan and Novy's "Cellular Toxins."¹

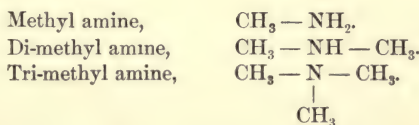
The ptomaines owe their basic character to nitrogen-containing radicals, principally amino-groups, and hence are formed from nitrogenous substances, chiefly proteids, which contain their nitrogen in the amino form. Probably most ptomaines arise from the decomposition of the proteid medium upon which the bacteria grow, although undoubtedly part of the ptomaines is also formed from the destruction of the bacterial cells themselves; how large a part of the ptomaines is formed by intracellular bacterial processes and how much by cleavage of the proteids of the media by extracellular bacterial enzymes is unknown. The structure of the ptomaines shows them to be very closely related to the amino-acids obtained by cleavage of the proteid molecule by enzymes and other hydrolytic agencies; hence it is probable that ptomaines are produced by secondary changes in the elementary nitrogenous "building stones" of the proteid molecule, the amino-acids. Presumably these secondary changes result from the action of special enzymes upon the amino-acids, *e. g.*, *urease* (a bacterial enzyme) splits urea into ammonia and carbon dioxide; but possibly they are partly due to interaction of the cleavage products upon one another. Most of the ptomaines are free from or poor in oxygen, hence reduction processes are probably important in their production. The poisonous ptomaines, which are decidedly in the minority among the entire group, are themselves subject to decomposition, being most abundant in the cultures after a certain period of time, and then decreasing in amount. Very old cultures show

¹ Philadelphia, 1902.

almost none of the higher molecular forms of nitrogen, such as ptomaïns, these substances having been changed into ammonium and nitrate compounds. In sharp contradistinction to the toxins, *the ptomaïns are by no means specific*. No matter upon what medium diphtheria bacilli grow, the toxin produced has qualitatively the same properties, whereas the nature of the ptomaïns depends not only upon the nature of the bacteria producing them, but also even more upon the sort of soil upon which the bacteria are grown, the temperature, the duration of the process, and the quantity of oxygen furnished. The same organism may produce totally different ptomaïns when grown on different media or under different conditions. Another essential difference is that we cannot obtain an immune serum, antagonizing the action of ptomaïns, by injecting ptomaïns into animals.

Ptomaïns are chiefly the cause of disease when they are taken in with food in which they have been produced by bacterial decomposition. Besides this food poisoning, it is also possible that ptomaïns may be formed by putrefaction within the gastrointestinal tract. Another possible source of ptomaïns is furnished by decomposing tissues in gangrene. It is doubtful if ptomaïns are produced in sufficient quantities by pathogenic bacteria infecting living tissue to be of any importance. Food-poisoning is by no means uncommon, but it is not always due to ptomaïns; it may be the result of poisonous materials contained abnormally in the food, that are not ptomaïns, *e. g.*, ergotism; or it may be due to an infection of the animal from which the meat came with pathogenic organisms, particularly the *B. enteritidis* of Gaertner and other bacteria related to the colon-typhoid group; or in other ways food ordinarily wholesome may become poisonous. The commonest sources of ptomaïn poisoning are imperfectly preserved canned meats, sausages, decomposing fish, cheese, ice-cream, and milk.¹

Chemical Composition of Ptomaïns.—To indicate the composition and nature of ptomaïns a few of the more important ones may be described. As illustrative of the simpler forms may be mentioned :

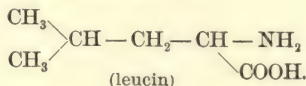
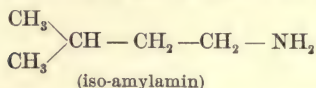


These bodies, which are commonly found in decomposing

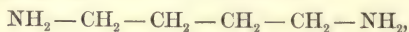
¹ All these matters are discussed at length by Vaughan and Novy, to whose book the reader is referred.

proteids, are but very slightly toxic, and of little pathological importance.

When we examine the structural formulæ of some of the larger ptomain molecules and compare them with the formulæ of the amino-acids that form the proteid molecule, the relation is apparent, *e. g.*, compare iso-amylamin with leucin.



Putrescin, $\text{C}_4\text{H}_{12}\text{N}_2$, structural formula,

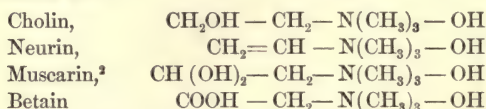


and *cadaverin*, $\text{C}_5\text{H}_{14}\text{N}_2$, structural formula,



are of interest because they have been found in the intestinal contents, arising from putrefaction of proteids, and also are sometimes present in the urine in *cystinuria*.¹ They are closely related to the diamino-acids, lysin and ornithin. They are but slightly toxic, although capable of causing local necrosis when injected subcutaneously. (See further discussion in Chapter xix.)

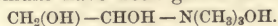
The Cholin Group.—Another group of ptomains, including cholin and closely related substances, is also of interest. These ptomains are:



The first point of importance is that cholin is present in every cell normally, forming the nitrogenous portion of the lecithin molecule. Its source in putrefaction of tissues is, therefore, plain. Furthermore, it seems to be liberated during life whenever nervous tissue, which is rich in lecithin, is broken down in any considerable amount. Mott and Halliburton³ claim that it can be found in both the blood and the cerebrospinal fluid of man and animals suffering with severe nervous lesions.⁴

¹ Udránszky and Baumann, *Zeit. physiol. Chem.*, 1889 (13), 562; 1889 (15), 77.

² Other structural formulæ have been given for muscarin, *e. g.*,



³ See Halliburton, "Chemistry of Muscle and Nerve," Philadelphia, 1904.

⁴ Coriat (*Amer. Jour. of Physiol.*, 1904 (12), 353) has studied the conditions under which cholin may be produced from lecithin. Putrefaction of lecithin or lecithin-rich tissues liberates cholin, as also does autolysis of brain tissue; neither pepsin nor trypsin, however, splits it from the lecithin. In brain tissue, therefore, there seems to be an enzyme different from trypsin, which splits cholin out of the lecithin molecule.

Cholin itself is somewhat toxic, but the closely related body, neurin, into which it may be transformed, is highly poisonous, which makes cholin an important indirect source of intoxication. It is possible, for example, that lecithin taken in the food splits off cholin in the gastro-intestinal tract, and this being converted into neurin gives rise to intoxication which may be ascribed to food intoxication. Likewise it has been suggested that the intoxication of fatigue may be due, at least in part, to cholin and neurin produced from lecithin decomposed during the period of cellular activity. The close structural relation to cholin and neurin, of the mushroom poison, muscarin, which produces physiological effects very similar to those of neurin, indicates the close relationship of the putrefactive ptomains and the vegetable alkaloids. Indeed a muscarin apparently identical with that of the mushroom has been found in decomposing flesh, and neurin, presumably derived from lecithin, may be found in human urine.¹ Betain, the fourth member of the group, which has but slight toxicity, is particularly well known as a constituent of plant tissues; possibly betain or other basic bodies may occur substituted for cholin in certain varieties of lecithin (Lippmann).

Both neurin and muscarin are extremely poisonous and quite similar in their effects. Subcutaneous injection of but 1 to 3 mg. of muscarin in man produces salivation, rapid pulse, reddening of the face, weakness, depression, profuse sweating, vomiting, and diarrhœa. Neurin, likewise, causes salivation, lachrymation, vomiting, and diarrhœa. In fatal poisoning respiration ceases before the heart stops. Both poisons resemble physostigmine in their stimulation of secretion and are equally well counteracted by atropin. The toxicity of these substances is so great that not a large amount would need to be formed by oxidation of cholin to produce severe symptoms, although it is not known that this actually occurs in the body. When introduced by mouth, the lethal dose of neurin is ten times as great as when injected subcutaneously, indicating that chemical changes in the gastro-intestinal tract offer some protection against intoxication by these substances when taken in tainted food. Cholin, although by no means so poisonous as neurin, has a similar action when administered in sufficiently large doses. According to Brieger, it is about one-tenth to one-twentieth as toxic as neurin.² Cholin seems to be rapidly destroyed in the body,

¹ Kutscher and Lohmann, *Zeit. physiol. Chem.*, 1906 (48), 1.

² Halliburton, "Chem. of Muscle and Nerve," 1904, p. 119, states that cholin produces a fall in blood pressure by dilating the peripheral vessels, whereas

not appearing in the urine¹ but forming formic acid and perhaps glyoxylic acid. Donath² found that cholin injected directly into the cortex or under the dura is extremely toxic, causing severe tonic and clonic convulsions, and believes that cholin may be responsible for epileptic convulsions, since he has found that cholin is present in the cerebrospinal fluid of epileptics. He corroborated the work of Mott and Halliburton, finding quantities large enough to detect (0.02 to 0.05 per cent.) in the cerebrospinal fluid of patients with dementia paralytica, tabes dorsalis, cerebral syphilis, brain abscess, and other conditions associated with destruction of nervous tissue, but in functional disorders he found it seldom or never. In genuine syphilitic and Jacksonian epilepsy cholin was found in 19 of 22 cases. Cholin may be found in normal cerebrospinal fluid, but in extremely minute quantities. When large nerves are cut, cholin appears in the blood, derived from the lecithin of the disintegrating nerve fibers, and is most abundant at the time the Marchi reaction is most prominent in the nerves.

TOXINS

Certain bacteria produce soluble poisons by synthetic processes, which poisons are secreted into the surrounding medium and which represent the chief poisonous products of the bacteria, being capable of causing most or all of the symptoms attributed to infection by the specific bacteria that have manufactured them. To this class of soluble poisons the term *toxin* has now become limited (for reasons that will be mentioned below), including not only toxins of bacterial origin, but also poisons of similar nature produced by animals (snake venoms, eel serum, etc.) and by plants (ricin, abrin, crotin). The bacteria secreting true toxins are *B. diphtheriæ*, *B. tetani*, *B. pyocyaneus*, and *B. botulinus* (not including bacteria producing hemolytic substances resembling toxins). It will be seen that the term toxin has been greatly narrowed since the time when all ptomaines and other poisonous bacterial products were called toxins, until

neurin constricts the peripheral vessels; he uses this difference in physiological effect as a means of distinguishing the two substances. Injected into animals, cholin causes a considerable but transient decrease in the number of leucocytes in the blood, followed later by an increase (Werner and Lichtenberg, Deut. med. Woch., 1906 (32), 22).

¹ v. Hoesslin, Hofmeister's Beitr., 1906 (8), 271.

² Zeit. f. physiol. Chem., 1903 (39), 526; also see Med. News, 1905 (86), 107, for literature and methods of analysis. Full review of subjects of cholin and neurin in these relations by Halliburton, Ergeb. der Physiol., 1904 (4), 23.

now it has come to include the specific poisons of but four of the great group of pathogenic bacteria.¹

Chemical Properties of Toxins.—The chemical nature of the toxins is entirely unknown. By various precipitation methods they may be carried down, but included with them are masses of impurities, chiefly proteids. It is quite certain that toxins are not proteids, since very active toxins have been obtained by purification processes that did not give the proteid reactions. The old name of "toxalbumin" is, therefore, incorrect. Oppenheimer² says of the toxins that "we must be contented to assume that they are large molecular complexes, probably related to the proteids, corresponding to them in certain properties, but standing even nearer to the equally mysterious enzymes with whose properties they show the most extended analogies both in their reactions and in their activities." These similarities between toxins and enzymes are very striking, and in discussing the nature of the enzymes we have mentioned the reasons for considering them related to the toxins; we may now take up the other side of the question and consider the relation of the toxins to the enzymes.

Resemblance to Enzymes.—First of all we meet the same difficulty in isolating toxins that we do in isolating enzymes. "A pure toxin is as unknown as a pure enzyme" (Oppenheimer). At first both were believed to be proteids; now both are considered by many not to be proteids, but molecular complexes of nearly equally great dimensions. That toxins, like enzymes, are colloids, has been abundantly demonstrated.³ Both pass through porcelain filters, but both lose much of their strength in the process, and they are almost entirely held back by dialyzing membranes. Neither will withstand boiling, and most forms are destroyed at 80° instantly or in a very short time; on the whole, however, toxins are more susceptible to heat, as well as to most other injurious agencies. Both stand dry heat over 100°, and extremely low temperature, without much injury. Left standing in solution for some time they gradually lose their specific properties, and in each case this seems to be due to an alteration in the portion of the molecule that produces the

¹ In addition to the ordinary toxins, Ehrlich recognizes other poisons secreted by the diphtheria bacillus which have a less specific and less actively poisonous action; and called *toxones*. (This conception is contested by Arrhenius and Madsen). The toxins also vary in their affinity for antitoxin, and on this basis have been divided into proto-, deuto-, and tritotoxin. These refinements of division are not necessary for our consideration of the chemical features of immunity.

² Kolle and Wassermann's Handbuch, 1903 (1), 351.

³ See Zangger, Cent. f. Bakt. (ref.), 1905 (36), 239.

destructive effects (*toxophore* or *zymophore* group), while the portion of the molecule that unites with the substance that is to be attacked (*haptophore* group) remains uninjured, the toxin becoming a *toxoid*, the enzyme a *fermentoid*. Enzymes as well as toxins are poisonous when injected into animals, and the animals react to each by producing substances (*anti-bodies*) that render each inert, probably in the same way. On the other hand, enzymes and toxins seem to produce their effects according to different laws:—A small amount of enzyme can in course of time produce an almost indefinite amount of effect, whereas toxins act more nearly quantitatively. It seems as if the enzyme were bound to the body upon which it acts, as is the toxin, but that after it has destroyed this body it is set free in a still active form, ready to accomplish further work, whereas the toxin is either not set free, or it becomes inactive after it has once been combined.

Agencies Destroying Toxins.—Toxins are very susceptible to light, direct sunlight soon destroying the power of toxin solutions.¹ Oxygen, even dilute as in air, is harmful; and all oxidizing agents, including oxidizing enzymes, destroy them quickly. Like enzymes, they withstand such antiseptics as chloroform, toluol, etc., and are precipitated by the heavy metals. Some agencies seem to attack only the *toxophore* portion of the molecule, *e. g.*, iodine, carbon disulphid (Ehrlich).

Introduced into the gastro-intestinal tract, most bacterial toxins are not absorbed (botulinus toxin excepted), cause no symptoms, and do not reappear in the feces; they are therefore destroyed by the contents of the tract, pepsin, pancreatic juice, and bile all being capable of destroying toxins.² They may, however, when injected subcutaneously, circulate unimpaired in the blood of non-susceptible animals, gradually disappearing, more through slow processes of destruction than by elimination. When injected into susceptible animals, they soon disappear from the blood, being fixed in the organs that they attack.

Differences from Ptomaines.—While ptomaines are formed by cleavage processes from the medium upon which the bacteria grow, and the same ptomaines can be produced by several different kinds of bacteria, the *toxins* are *synthetic products of absolutely*

¹ Fluorescent substances have a destructive effect upon toxins, even in the animal body, according to Iodlbauer and v. Tappeiner, *Deut. Arch. klin. Med.*, 1905 (85), 399.

² Baldwin and Levene (*Jour. Med. Research*, 1901 (6), 120) found that diphtheria and tetanus toxin are both destroyed, apparently through digestion, by pepsin, trypsin and papain acting for several days. Review of Literature by Lust, Hofmeister's Beitr., 1904 (6), 132.

specific nature. That they are produced by synthesis can be shown by growing the bacteria on Uschinsky's or similar media, which contain no proteids, carbohydrates, or fats, but merely simple organic and inorganic salts of known composition; on these media the bacteria produce their specific toxins, which must, therefore, be synthesized.¹ Furthermore, diphtheria toxin is essentially the same no matter on what sort of medium it is grown, whereas ptomaines vary with the nature of the substance from which they are produced. Toxins are true secretions of bacterial cells, just as trypsin is of pancreatic cells, or thyroiodin of thyroid cells. Anti-bodies can be produced against toxins, but not against ptomaines.

Ehrlich's Conception of the Nature of Toxins.—

Chemical studies of toxins being impossible, we have been obliged to study them through their physiological effects, just as we have obtained information concerning enzymes through their specific actions. In this way Ehrlich has obtained well-crystallized ideas concerning the structure of toxins, as well as the manner in which they act, which may be briefly summarized as follows: Each toxin molecule consists of a large number of organic complexes grouped, as in other organic compounds, as side-chains about a central chain or radical. One or more of these complexes has a chemical affinity for certain chemical constituents of the tissues of susceptible animals, with which the toxin molecule unites; this binding group is called the *haptophore* (meaning "bearing a bond"). Another side-chain or group of side-chains exerts the injurious effects upon the tissue to which the molecule has been bound by the *haptophore*, and cannot produce these injurious effects unless it has been so bound. This injury-working group is called the *toxophore*. An animal is susceptible to a toxin only when its cells contain substances which possess a chemical affinity for the *haptophore* groups of the toxin, and also substances which can be harmfully influenced by the *toxophore* groups. Tetanus toxin, for example, owes its effects to the fact that nervous tissues contain chemical substances having a strong affinity for the *haptophore* group of tetanus toxin, and also substances that can be attacked with serious results by the *toxophore* group of the toxin. The nature of the changes brought about by the *toxophore* groups of toxins is not understood; there are many resemblances to the action of enzymes, but the analogy is by no means complete. We find perhaps the closest analogy to the enzymes in the toxic substances that destroy red corpuscles and bacteria (*hemolysins*,

¹ Zinno could not confirm this observation (Cent. f. Bakt., 1902 (31, Abt. 1), 42).

bacteriolysins), which will be considered in another place. The immunity against toxins and enzymes seems to be produced by identical processes, which consist in an overproduction of the cellular constituents (*receptors*) which bind the haptophore groups to the cells, these excessive receptors being secreted into the blood, where they combine with the toxin or enzyme so that it cannot enter into combination with the cells.

Immune substances cannot be produced against ptomaines, or for that matter against the vegetable alkaloids, or against any chemical bodies of known constitution. Another difference between the action of toxins and simpler chemical poisons is, that while with the latter the effects are produced in a very short time after injection, there is a *latent period* of several hours before symptoms appear after injecting toxins. What occurs during this latent period is not fully known, but that there is a latent period suggests a resemblance to enzyme action. An alkaloidal or other chemical poison enters the cell, and its harm is done at once. A toxin combines with the cell, and then, if it produces its effects by an enzymatic alteration of the cellular structure, some time must elapse before the changes are great enough to cause the appearance of symptoms.

ENDOTOXINS

By far the greater number of pathogenic bacteria do not secrete their poisons as toxins into the surrounding medium, although they manifestly cause disease by poisoning their host. Among them are such organisms as the typhoid bacillus, pneumococcus, the pus cocci, cholera vibrios, and many others. If cultures of these organisms are filtered, the filtrate will be found to be but slightly toxic (except for the hemolytic poisons), although the bodies of the bacteria after they have been killed by chloroform or other antiseptics are highly poisonous if injected into an animal. These bacteria, then, produce poisons which do not escape from the cells into the culture-medium, but are firmly held within them. By using various means these intracellular toxins, or *endotoxins*, can be obtained independent of the bacterial cells. One of these is to grind up the cells, which can be particularly well done if they are first made brittle by freezing at the temperature of liquid air (MacFadyen's method). By very great pressure in the Buchner press the cellular contents can be expressed. They may also be obtained by letting the bacteria autolyze themselves for a short time in non-nutrient fluids (Conradi,¹ *et al.*). Endotoxins obtained in this way are

¹ *Loc cit.*

soluble and highly poisonous, and it is undoubtedly through their action that the characteristic diseases are produced by the bacteria that contain them. Presumably the endotoxins are liberated in the body either by autolysis, or, more probably, by heterolysis by the enzymes of the body cells and fluids.

Endotoxins differ from the true toxins, however, in one important respect: namely, *no antitoxin has been obtained for endotoxins by immunization of animals.*¹ Animals immunized against endotoxins develop in their serum substances that are bactericidal and agglutinative to the bacteria from which the poisons are derived, but the serum will not neutralize the endotoxins.² As a result, we are unable to perform experiments indicating whether endotoxins have the same structure as the true toxins, *i. e.*, a haptophore and a toxophore group, but presumably their nature is different in some essential particular. The chemical nature of the endotoxins is also unknown, for they are always obtained mixed with the other constituents of the bacteria.

Since far more bacterial diseases are brought about by endotoxins than by true toxins, the failure to secure antitoxins for these substances has been a great check in the progress of serum therapy, and the problem of the endotoxins is one of the most important in the entire field of immunity.

POISONOUS BACTERIAL PROTEIDS

If we filter a bouillon culture of diphtheria bacilli through porcelain, wash thoroughly the bacteria remaining with salt solution, and collect them thus freed from their secretion products, it will be found that extracts of the bacterial substance or the bodies of the killed bacteria themselves are quite free from the typical toxin. This indicates that the toxin is eliminated from the bacteria as fast as it is formed, and no considerable quantity is retained within the cell. The bacterial substance, however, or proteids isolated from it, is found to produce severe local changes when injected into the bodies of animals, necrosis and a strong inflammatory reaction with pus-formation being the chief features. This local effect is not a specific property of the diphtheria bacillus, for other bacterial proteids, including proteids from non-pathogenic bacteria, will produce the same changes; indeed, many proteids that are derived from vegetable and animal sources have equally marked pyogenic properties. All foreign proteids when introduced into the circulation of animals

¹ Positive results are claimed by Besredka, (Ann. Inst. Pasteur, 1906 (20), 304), and a few others; see Kraus, Wien. klin. Woch., 1906 (19), No. 22.

² See résumé by F. Schmidt, Zeit. f. Infektionskr. der Haustiere, 1906 (1), 238, and Hahn, Münch. med. Woch., 1906 (53), No. 23.

are more or less toxic, and the toxic effects of the bacterial proteids are, for the most part, neither specific nor particularly striking. There are a few pathogenic organisms, however, which seem to produce neither true toxins nor endotoxins, notably the tubercle bacillus and the anthrax bacillus, and with these there may be a relation between their proteid constituents and their specific effects.¹

Numerous proteid substances have been extracted from bacterial cells, particularly nucleoproteids, but also proteids resembling albumins, nucleo-albumin, and globulins. In all probability the chief proteids of the bacterial cell are nuclein compounds, which is indicated both by their nuclear staining and by the analyses of Iwanoff;² and many of the nucleoproteids, both of bacterial and non-bacterial origin, cause considerable local inflammatory reaction when injected into animals. Tiberti³ claims that vaccination with non-lethal doses of the nucleoproteids of anthrax bacilli will protect animals against inoculations of virulent anthrax bacilli. Some of the earlier observations on the toxicity of bacterial proteids were erroneous because impure proteids, containing toxins, endotoxins, and ptomaines were used.

Vaughan and his students have been able to split off from the bodies of various pathogenic bacteria toxic materials which are stated to resemble in some respects the protamins,⁴ although they do not all give a satisfactory biuret test. These toxic materials are evidently quite different from either the true soluble toxins or the endotoxins, since they resist heating for ten minutes, at 110° in the autoclave with 1 per cent. sulphuric acid, this being the method used for securing the substance, which is precipitated out by alcohol. Since the sarcinæ and *B. prodigiosus* also yield similar toxic products, they cannot be considered as the specific toxic substances of the pathogenic bacteria. With some bacteria the splitting process with sulphuric acid separates completely the toxic from the non-toxic insoluble bacterial substance,⁵ e. g., *B. coli communis*; with others a toxic portion remains insoluble. The colon bacillus proteid gives all the proteid reactions, is synthesized on Uschinsky's medium, and does not yield a reducing carbohydrate. From *B. typhosus*

¹ Baldwin and Levene (*loc. cit.*) found that the active constituent of tuberculin was destroyed or digested by trypsin and not by pepsin, indicating that it was probably a nucleoproteid.

² Hofmeister's Beitr., 1902 (1), 524.

³ Cent. f. Bakt., 1906 (40), 742.

⁴ Jour. Amer. Med. Assoc., 1903 (40), 838; 1904 (43), 643; see also Boston Med. and Surg. Jour., Aug. 30 *et seq.*, 1906.

⁵ Wheeler, Jour. Amer. Med. Assoc., 1905 (44), 1271.

about 10 per cent. by weight of proteid can be split off by dilute acid, of which at least a part seems to be a phosphorized glycoproteid.¹ Poisonous substances have also been obtained from *B. diphtheriae*, *B. anthracis*, and *B. pyocyaneus*. They produce death without the usual latent period observed with toxins, but are very toxic, a few (10–20) milligrams of colon bacillus poison killing guinea-pigs in less than ten minutes.² A certain degree of immunity can be obtained against them.³ Their relation to endotoxins has yet to be determined.⁴ It is possible that they are toxic bodies derived from the endotoxins through alterations produced during the process of isolation, bearing the same relation to endotoxins that acid and alkali albuminate do to the original proteids—modified or “*denaturierte*” proteids (Wolff⁵).

BACTERIAL PIGMENTS⁶

The formation of pigment by bacteria seems to be, for the most part, an adventitious, unessential property. There are a few bacteria which possess pigments of the nature of chlorophyll, or allied to it, and this pigment is undoubtedly of great importance in the life processes of these particular forms. Other varieties of pigment-forming bacteria, of which but very few are pathogenic (*Bacillus pyocyaneus*, *B. proteus fluorescens*, *S. pyogenes aureus* and *citreus*, *M. cereus flavus*), seem to produce pigment as a waste product which is excreted from the cell as fast as formed. Generally the pigments are produced in a colorless form (*leuco-base*) which is oxidized by the air into the pigment, *e. g.*, in pyocyaneus infections the soiled dressings are most colored about the portions most exposed to air. Since pigment-forming bacteria produce pigments only under certain conditions, and can grow abundantly without producing any pigment, it is evident that the pigment formation is no very essential part of their metabolism. It is possible to modify pigment production almost at will, and even to develop races of bacteria that do not produce pigment at all, from races that ordinarily are pigment-producers.

Of numerous classifications of pigment-forming bacteria, all

¹ *Ibid.*, 1904 (42), 1000.

² *Ibid.*, 1905 (44), 1340; American Medicine, 1905 (10), 145.

³ Vaughan (Jr.), Jour. of Med. Research, 1905 (14), 67.

⁴ An important argument against the specific nature of these poisons is the close resemblance to poisons obtained from liver cells, egg-albumen, etc., by similar methods. Vaughan considers that every protein molecule, whether bacterial or not, has a poisonous group, which contains the benzene ring.

⁵ Cent. f. Bakt. (1 Abt.), 1904 (37), 687.

⁶ For complete bibliography and résumé see Sullivan, Jour. Med. Research, 1905 (14), 109.

faulty because of our slight knowledge of the chemistry of the process, that of Migula seems the best; it is based on the solubility of the pigments formed, as follows:

(1) **Pigments Soluble in Water.**—This includes the pigments of all fluorescent bacteria, as well as those giving a red or brown color to gelatin media. Most important among these is *Bacillus pyocyaneus*, whose pigments have been considerably studied. There seem to be two pigments, one, *pyocyanin*, characteristic for this organism; and a fluorescent pigment which numerous other organisms also produce. Pyocyanin has been analyzed by Ledderhose, who found it to be a ptomain-like body, a derivative of the aromatic series, probably related to the anthracenes. It can be reduced to a colorless leuco-base, in which form it is probably produced by the bacteria, and then is oxidized in the air into the pigment. Its composition is $C_{14}H_{14}N_2O$ (the sulphur-containing pyocyanin which has been described is probably impure). The fluorescent pigment is insoluble in alcohol and chloroform, and can thus be separated from pyocyanin, which is soluble in chloroform. Although related to the ptomains, pyocyanin seems to be altogether non-poisonous to animals.

Jordan¹ and Sullivan² have studied the conditions under which pigments are formed, and found that pyocyanin can be produced in proteid-free media, and without the presence of either phosphates or sulphates; but both sulphur and phosphorus must be present to produce the fluorescent pigment. As pigments can be produced on media containing ammonium salts of succinic, lactic, or aspartic acid, or asparagin, they are evidently formed synthetically, and not by cleavage of the media.

(2) **Pigments Soluble in Alcohol and Insoluble in Water.**—The most important bacteria of this group are the *Staphylococcus pyogenes aureus* and *citreus*. Their pigment is of a fatty nature, a *lipochrome*, which lies among the bacteria in the form of dendritic crystals. Being a fat, it can be saponified, and when decomposed it gives the acrolein reactions and odor, from the breaking down of the glycerin of the fat molecule. Acted upon by strong sulphuric acid, the yellow pigment changes into blue granules and crystals (*lipocyanin* reaction). The lipochromes are soluble in the usual fat solvents, and form fat spots on paper.

(3) **Pigments Insoluble in Water and in Alcohol.**—The pigment of *Micrococcus cereus flavus* belongs to this class; its nature is quite unknown.

¹ Jour. Exper. Med., 1899 (4), 627.

² Loc. cit.

CHAPTER V

CHEMISTRY OF THE ANIMAL PARASITES¹

THIS subject has received much less consideration than its importance deserves, and we are quite in the dark as to how much of the effects produced by animal parasites are not merely mechanical, but are due to soluble poisons that they may secrete or excrete. Some of the parasites probably cause harm mechanically and in no other way, but with most of them there is more or less evidence of the formation of poisonous substances. The composition of the bodies of the animal parasites is an almost unexplored field, but we have no reason to believe that the composition of the cells of invertebrates differs essentially from that of the cells of higher organisms. Perhaps the most characteristic constituent observed in many forms is *chitin*, which forms a large part of the outer covering of the encysted forms, and probably of many of the worms. *Glycogen* is usually abundant in the invertebrates, and the animal parasites form no exception,² this carbohydrate having been found in their bodies by many observers.

Eosinophilia.—One of the most characteristic features of the animal parasites is that they exert a positive chemotaxis, particularly for eosinophile leucocytes.³

An increase in the number of these cells in the blood, as well as a local accumulation in the tissues nearest the parasite, has been observed in infection with the following parasites:⁴ *Uncinaria duodenalis*, *Strongyloides intestinalis*, *Ascaris lumbricoides*, *Tænia solium*, *Tænia saginata*, *Tænia echinococcus*,⁵ *Filaria bancrofti*, *Bilharzia hæmatobia*, *Trichinella spiralis*, and *Amœba coli*.⁶ Of these, infection with *Trichinella spiralis* causes the most pronounced eosinophilia, presumably because of the great

¹ General references to this subject will be found in v. Fürth's "Vergleichende chemische Physiologie der niederen Tiere," Jena, 1903; and Faust's "Tierische Gifte," Braunschweig, 1906.

² See Pflüger, Pflüger's Arch., 1903 (96), 153.

³ Literature by Opie, Amer. Jour. Med. Sci., 1904 (127), 477; and Stäubli, Dent. Arch. klin. Med., 1906 (85), 286.

⁴ Literature by Bruns, Liefmann and Mäckel, Münch. med. Woch., 1905 (52), 253.

⁵ See Dévé, Compt. Rend. Soc. Biol., 1905 (59), 49.

⁶ Billet, Semaine méd., 1905 (25), 261.

number of parasites present in the tissues at once. That the eosinophilia is due to the action of the soluble products or constituents of the parasites has been shown by experimental injection into animals of extracts from the bodies of the parasites.¹ Calamida has found that extracts of dog tapeworms also, when placed in the tissues in a capillary tube, cause an accumulation of eosinophile cells in the tube. Experimental infection with excessive numbers of trichinella causes a rapid diminution in the number of eosinophile leucocytes, which also show evidences of disintegration in the bone-marrow and lymph-glands. Such large injections are fatal, which suggests that the eosinophilia has a protective influence. In favor of this view is the observation of Milian,² who found that sarcosporidia in beef are destroyed by a violent leucocytic reaction, the prevailing cell being the eosinophile. As the eosinophile increase does not occur until several days after the infected flesh is eaten, the chemotactic substance is not liberated from the encapsulated trichinellæ when their capsules are digested off in the gastric juice, but comes either from the free larvæ, or from the degenerated muscles in which they burrow. Coincident bacterial infection may reduce the number of eosinophiles.

PROTOZOA

These unicellular forms possess all the chemical characters of the cells of higher forms, even to the more specialized constituents. Thus it has been demonstrated that protozoa contain proteolytic enzymes,³ and that they secrete an acid into their digestive vacuoles.⁴ On the other hand, *Amæba coli* does not seem to digest the red corpuscle and the bacteria that it takes up.⁵ Whether the *Amæba coli* produces any toxic materials, specific or non-specific, has not yet been determined, but the necrosis that it produces in liver abscesses, when bacterial co-operation can often be excluded by culture, strongly indicates the production of necrogenic substances. Apparently these substances are not chemotactic, in view of the absence of leucocytic accumulation which is characteristic of the lesions of amebic

¹ If Habershon's views (Jour. Pathol. and Bacteriol., 1906 (11), 95) on the relation of glycogen to the eosinophile granules is correct, it is possible that there exists some relation between the abundance of glycogen in the animal parasites and their tendency to cause eosinophile accumulations.

² Bull. et Mem. Soc. Anat., 1901 (Ser. 6, T. 3), 323.

³ Mouton, Compt. Rend. Soc. Biol., 1901 (53), 801.

⁴ Le Dantec, Ann. Inst. Pasteur, 1890 (4), 776; Greenwood and Saunders, Jour. of Physiol., 1894 (16), 441.

⁵ Musgrave and Clegg, Bureau of Gov't. Laboratories, Manila, 1904, No. 18, p. 38.

dysentery. There is also no evidence, clinical or experimental, that amebic infection causes the formation of anti-substances of any kind in the body of the host. The spontaneous recovery from amebic and other protozoan infections, however, may be considered as indicating the development of an *immunity* against these organisms. Numerous observers have suggested the possibility of obtaining artificial immunity against protozoa, and Rössle¹ has obtained immune sera against infusoria.

Plasmodium malarie undoubtedly produces toxic substances, which seem to be of such a nature that they do not diffuse from the red corpuscle, but are only liberated when the corpuscle breaks up on the maturation of the parasite. In this way the characteristic paroxysmal manifestations of the disease are produced. The nature of the poison or poisons is unknown, but we have evidence that it is hemolytic, since malarial serum may hemolyze normal corpuscles.² Presumably it is not extremely toxic for parenchymatous cells, since the parenchymatous lesions in malaria seem to be relatively slight as compared with the intensity and duration of the intoxication. Some authors state that the toxicity of the urine is increased after the paroxysm,³ which, however, does not necessarily indicate that a poison formed by the parasites is excreted in the urine. Immunity seems to be seldom developed against the malarial poison or against the parasite itself, although some persons seem to be naturally immune, while some acquire immunity through previous infection.⁴ Many writers have looked upon the pigment present in the malarial parasites as a true melanin, produced by their metabolism and not a product of decomposition of hemoglobin; however, Ewing⁵ found that it showed the same relation to solvents as the blood-pigments (See "Pigmentation," Chapter xvi).

Sarcosporidia of sheep (*Balbiania gigantea*, Railliet) yield aqueous and glycerin extracts that are highly toxic for rabbits (Pfeiffer), the poisonous constituent of which was called *sarcocystin* by Laveran and Mesnil.⁶ This is so highly toxic that 0.0001 gm. is fatal to rabbits (per kilo), other animals being less susceptible. It loses its toxicity on heating at 85°

¹ Arch. f. Hyg., 1905 (54), 1; full review of this topic.

² See Regnault, Revue de Méd., 1903 (23), 729.

³ Quoted from Blanchard, Arch. d. Parasitol., 1905 (10), 83; this article gives a résumé of the subject of the toxic substances produced by the animal parasites.

⁴ See Celli, Cent. f. Bakt., 1900 (27), 107.

⁵ Jour. Exper. Med., 1902 (6), 119.

⁶ Compt. Rend. Soc. Biol., 1899 (51), 311.

for twenty minutes, and is somewhat impaired at 55–57° for two hours. It is probable that the pathogenic protozoa, at least in some instances, have a semipermeable membrane about them, for Goebel¹ found that trypanosomes are very susceptible to changes in osmotic conditions.

CESTODES

Tænia echinococcus has been by far the most studied, its abundant fluid content furnishing suitable material for investigation. That this fluid is toxic has been repeatedly observed when, through rupture or puncture, the fluid has escaped into the body cavities; such accidents are often followed by violent intoxication, sometimes by death.² The most constant symptoms are local irritation and inflammation, accompanied by urticaria, which may also be produced experimentally in man if the cyst contents are injected subcutaneously. The fluid is also highly toxic to many animals. As long as the cyst is unopened no toxic manifestations are observed, presumably because the toxic substances do not diffuse through the cyst wall. The nature of the toxic substances is not known, although Brieger isolated a platinum salt of a substance that killed mice.

The *fluid* of the echinococcus cysts has generally a specific gravity of 1005–1015, and contains 1.4–2 per cent. of solids. Most abundant are sodium chloride, about 0.8 per cent., and sugar, 0.25 per cent., the latter presumably coming from the glycogen contained in the wall. Cholesterin is often abundant, while inosite, creatin, and succinic acid are often found. Clerc has found traces of lipase, but other enzymes seem to be absent or in very small amounts. Proteids are present only in traces, unless inflammation has occurred. Schilling³ found the molecular concentration of the cyst fluid to be quite the same as that of the patient's blood.

The *cyst wall* consists of a hyaline substance which seems to stand between the chitin and the proteids, and probably consists of a mixture of both. Because of the chitin it yields about 50 per cent. of a reducing, sugar-like body when boiled with acids. Glycogen is also usually present, but it is limited to the germinating membrane.⁴

Other cestodes, when in the cystic form, contain fluids which are more or less toxic. Thus Mourson and Schlagdenhauffen⁵

¹ Ann. Soc. Méd. d. le Gand, 1906 (86), 11.

² See Achard, Arch. gén. de Méd., 1887 (22), 410 and 572.

³ Cent. inn. Med., 1904 (25), 833.

⁴ Brault and Loeper, Jour. Phys. et Path. gén., 1904 (6), 295.

⁵ Compt. Rend. Soc. Biol., 1882 (95), 791.

found a "leucomain" in the *Cysticercus tenuicollis*, the larva of *Tenia marginata*, which causes urticaria and other toxic symptoms when injected into animals. The fluids of *Cysticercus pisiformis* (the common cestode of rabbits) have been found toxic for frogs, and Vaullegeard¹ has determined the presence of an "alkaloid" and a "ferment toxin" in this fluid. The fluids of the cysts of *Cœnurus cerebralis*, *Cœnurus serialis*, and *Echinococcus polymorphus* have all been found toxic, and it is probable that this is a general rule with the cestodes,² but human forms other than the echinococcus seem not to have been investigated;³ according to Jammes and Mandoul, extracts of tænia are bactericidal.

Dibothriocephalus latus frequently causes anemia, which has been attributed to a poison liberated by the parasite when it undergoes disintegration, and possibly as a secretion of the living worm.⁴ All the intestinal cestodes are equipped with a well-developed excretory apparatus, and it is easy to imagine that their excretory products may be toxic to the animal into whose intestine they are excreted. Schauman and Tallqvist⁵ found that extracts from these worms were toxic to dogs however administered, and caused a marked anemia; in the test-tube these extracts were hemolytic.

Rosenqvist⁶ has studied the metabolism of twenty-one cases of bothriocephalus anemia, and found evidence in nearly all of a toxogenic destruction of proteid, which ceases promptly when the worms are removed. He has found that these worms produce a poison which is globulicidal, and probably also generally cytotoxic, since in the anemias that they produce, the elimination of purin bodies of tissue origin (endogenous purin) is increased. The nitrogenous metabolism is quite the same in pernicious anemia and in bothriocephalus anemia. Isaac and v. d. Velden⁷ state that the blood of patients infected with this parasite gives a *precipitin reaction* with autolytic fluid obtained from bothriocephalus, and that rabbits immunized with such autolytic fluids developed a precipitin.

Other Tænia.—There is much less evidence that other forms of tænia produce toxic substances which injure their host, although the clinical manifestations observed in persons harboring tænia are often of such a nature as to indicate strongly an intoxication. Jammes and Mandoul⁸ found no toxic manifesta-

¹ Bull. Soc. linnéenne de Normandie, 1901 (4), 84.

² Blanchard, *loc cit.*

³ Semaine méd., 1905 (25), 55.

⁴ Literature by Blanchard, *loc cit.*

⁵ Deut. med. Woch., 1898 (24), 312.

⁶ Zeit. klin. Med., 1903 (49), 193.

⁷ Deut. med. Woch., 1904 (30), 982.

⁸ Compt. Rend. Acad. Sci., 1904 (138), 1734.

tions produced by extracts of *Tænia saginata*, which negative finding is supported by Cao,¹ and by Boycott,² using various sorts of tænia. These results contradict the earlier positive findings of Messineo and Calamida,³ who found extracts of tænia from dogs to be hemolytic, chemotactic (especially for eosinophiles), and to cause local fatty degeneration in the liver. Possibly these differences in results are due to the fact that different parasites were studied by different investigators; furthermore, tests of toxicity of human parasites upon rabbits and guinea-pigs can hardly be considered conclusive. Le Dantec did not find a precipitin for *Tænia saginata* extracts in the blood of persons harboring this parasite.

Picou and Ramond⁴ state that tænia extracts undergo putrefaction very slowly, and attribute this to a bactericidal property. Weinland⁵ has found that many intestinal parasites exhibit *antitryptic* properties, but in a study of the histological changes of autolysis I observed a tænia in the intestine of a dog undergo more rapid karyolytic changes than did the intestinal epithelium. Dastre and Stessano⁶ state that extracts of *Tænia serrata* act upon enterokinase, rather than on trypsinogen.

NEMATODES

Ascaris.—The toxicity of members of this group is a matter of dispute, although, as with the *Tænia*, there have been observed in patients symptoms that were more easily explained as due to chemical substances than as due to mechanical irritation. Miram,⁷ while studying *Ascaris megalocephala*, suffered from attacks of sneezing, lachrymation, itching, and swelling of the fingers. v. Linstow suffered from a severe attack of conjunctivitis with chemosis after touching his eye with a finger that had been in contact with one of these worms. Others have had similar experiences, and it has been found that the fluid from these worms is toxic to rabbits (Arthus and Chanson,⁸ Vaullegeard⁹). Blanchard, nevertheless, considers that the toxic manifestations observed in patients infected with these worms are most probably due to bacterial infection of the injured intestinal mucosa. Jammes¹⁰ found

¹ Riforma med., 1901 (3), 795.

² Jour. Pathol. and Bacteriol., 1905 (10), 383.

³ Cent. f. Bakt., 1901 (30), 346 and 374.

⁴ Compt. Rend. Soc. Biol., 1899 (51), 176.

⁵ Zeit. f. Biol., 1902 (44), 1 and 45.

⁶ Compt. Rend. Soc. Biol., 1903 (55), 130.

⁷ Quoted by Nuttall, Amer. Naturalist, 1899 (33), 247.

⁸ Cent. f. Bakt., 1896 (20), 264.

⁹ Quoted by Blanchard, *loc cit.*, p. 98.

¹⁰ Assoc. française pour l'avancement des sciences, 1902 (31), 241.

that *A. lumbricoides* and *Oxyuris vermicularis* do not produce toxic materials, and Boycott¹ obtained a negative result with extracts of *A. lumbricoides* filtered through porcelain to exclude bacteria. Allaria² also obtained negative results, and could demonstrate no hemolytic properties. On the other hand, Cattaneo³ claims that filtered culture-media in which *Ascaris* has lived for some time is toxic for guinea-pigs.

Weinland first demonstrated that *Ascaris* and other intestinal worms are able to live in the digestive fluids of the intestine because they contain an active *antitrypsin*. Dastre and Stassano considered the active agent an antikinase, but Weinland's view has been confirmed by Hamill.⁴

Glycogen has been found abundantly in ascaris, and chitin⁵ is present in the external covering of some forms. Reichard⁶ found that in *A. lumbricoides* and *A. sipunculides* the cuticle is formed of an albuminoid material, but in the *Hirudines* it is a true chitin.

Trichinella spiralis unquestionably produces toxic substances, as shown by the profound intoxication and febrile condition of persons suffering from infection with this parasite. As to the nature of the poison, however, we know nothing, except that it causes cellular degeneration, and is particularly chemotactic for eosinophiles.

Uncinaria duodenalis, which has for its chief effect the production of a severe anemia, seems to cause this anemia by producing repeated small hemorrhages rather than by any toxic action. The abundance of this loss of blood is explained by L. Loeb⁷ as due to the presence, in the anterior portion of the parasite (*Ankylostoma caninum*), of a substance that inhibits the coagulation of the blood, analogous to the "hirudin" of the leech.

Filaria seem not to produce any appreciable amount of toxic material, if we may judge by the slight evidence of intoxication shown by infected individuals. An exception may be made in the case of the guinea-worm (*Dracunculus* or *F. medinensis*). This parasite causes chiefly mechanical injury unless its body is ruptured, which may happen in attempting to remove it forcibly; this accident is followed by violent local inflammation or gangrene, which indicates that some powerfully irritant substance is liberated from the torn body of the worm.

¹ *Loc. cit.*

² Ref. in Cent. f. Bakt., 1905 (35), 539.

³ Ref. in Biochem. Centr., 1903 (1), 806.

⁴ Jour. of Physiol., 1906 (33), 479; literature.

⁵ Weinland, Zeit. f. Biol., 1902 (43), 86.

⁶ "Ueber Cuticular- und Gerüst-substanzen bei wirbellosen Tieren," Heidelberg, 1902.

⁷ Cent. f. Bakt., 1904 (37), 93; 1906 (40), 740.

CHAPTER VI

CHEMISTRY OF IMMUNITY AGAINST BACTERIA AND THEIR PRODUCTS, AND THE REACTIONS OF AGGLUTINATION AND PRECIPITATION¹

By the application of chemical principles to the problems of immunity, Ehrlich developed a hypothesis concerning the nature of the action of bacterial toxins upon the cells, and of the process of antitoxin formation, which has served most successfully as a working hypothesis. The true toxins are of so labile a nature, so readily destroyed by chemical agencies, and so elusive of isolation, that their chemical natures and properties remain quite unknown, and they can be detected only by their biological action. Against other sorts of poisons with simpler composition the animal body does not develop an immunity in the same sense that it does against bacterial and similar poisons, and so in studying the reactions of immunity we cannot have the advantage of having at least one of the factors a substance of known chemical nature. By immunization or habituation a certain degree of resistance can be obtained against some alkaloidal poisons, *e. g.*, morphine, but it is not of the same nature as the immunity against bacterial toxins, for the blood-serum does not acquire any substances capable of neutralizing the poisons. The resistance against such poisons must, therefore, be considered apart from the question of immunity against bacterial infection (see Chapter vii); but with the latter may be included the consideration of immunity artificially developed in the body against foreign proteids, tissues, and cells.

Immunity against bacteria may be divided into several subjects, namely, immunity against bacterial toxins, against bacterial proteids and enzymes, and against the bacteria themselves, including the phenomenon of agglutination. The products

¹ Only the chemical processes and principles underlying the defense of the body against bacterial and other poisons will be discussed in this chapter. For a consideration of the general features of immunity see "Infection, Immunity, and Serum Therapy" by Ricketts, Chicago, 1906. For bibliography see Kolle and Wassermann, 1903, Bd. 4. Later references of importance have been cited in the foot-notes of this chapter.

formed by bacterial decomposition of proteids, the ptomaines, do not give rise to immune substances.

TOXINS AND ANTITOXINS

The first phase of immunity to be considered is the neutralization of toxin by antitoxin, since it is the most complete and best understood of the reactions of immunity. In the preceding chapter on the bacteria and their products the nature of the *true toxins* was defined, and attention was called to the fact that one of their most important characteristics is that immunization of animals against them leads to the accumulation in the blood of substances capable of completely neutralizing their poisonous action. Such true toxins are produced especially by the diphtheria bacillus and the tetanus bacillus, also, but less strikingly, by *B. pyocyaneus*, *B. botulinus*, and possibly by a few others. In addition to these, numerous bacteria produce *hemolytic poisons* which seem to have properties similar to the toxins; and there are also toxins produced by plants (abrin, ricin, croton, and mushroom poisons) and by animals (snake venom, scorpion and spider toxin, and eel serum). Against all of these, true antitoxins may be obtained by the immunization of animals.

Ehrlich's Conception of Toxins and Antitoxins.—

According to Ehrlich's theory, the action of toxins upon cells is purely chemical. A toxin unites with a cell because some chemical group in the molecule of toxin has a chemical affinity for some particular group in the cell protoplasm. For convenience in description names have been given to these groups; the group of the toxin that combines with the cell has been called the *haptophorous* group, or *haptophore*, while the group in the protoplasm that combines with the toxin is known as the *receptor*.¹ It has been found that after being kept for some time, or when placed under certain unfavorable conditions, the toxin loses its poisonous properties without losing its power to combine with cells, as shown by the fact that immunization with such altered toxin gives rise to the formation of antitoxin.

¹ Ehrlich has used certain diagrams to illustrate these various groups and their relations to the cells and to one another, which are generally used in explaining his theory. From a teaching standpoint they have seemed to be undesirable, in that the student soon comes to ascribe physical properties and appearances to what should be considered as chemical combinations. The toxophore group becomes "the black fringed end of the toxin," etc. To one accustomed to thinking in chemical terms there is no difficulty in following the literature and understanding the reactions as chemical reactions, which they are.

Therefore it is not the haptophore that causes the harm to the cell, but there must be some other group with this particular function. To the group that produces the harm the name *toxophore* is given. If all the receptors of a cell are combined by toxin molecules that have lost their toxophore group (*toxoid* is the name given to such altered toxins), the cell cannot then be injured by the corresponding active toxin, showing that the toxin must first become united to a cell receptor by its haptophore group before the toxophore group can cause an injury.

Animals that are naturally immune to toxins may owe their immunity to the fact that their vital tissues contain no substances with a chemical affinity for the toxin, and hence the toxin cannot unite with them to cause harm. (In Ehrlich's terminology, the cells contain no receptors for the toxin.) The toxin may not combine with any tissue element at all in such immune animals, and circulate for some time harmlessly in the blood; or it may combine with some organ where it does little harm, *e. g.*, tetanus toxin is said to combine chiefly in the liver of some animals, and therefore it does not harm their nervous system.

According to this theory, *the antitoxin consists of cell receptors that have been produced in excess and secreted by the cells into the blood*. In the blood they combine with any toxin that may have been introduced, and by saturating its affinities render it incapable of uniting with the cells. As the toxin harms cells only after it has been chemically united to them, it is rendered harmless when its affinities for the cell (the haptophore groups) are saturated by cell receptors in the blood stream. The process of immunization consists in injuring the body cells to such a degree that they are stimulated to regenerate the receptor groups with which the toxin combines; these receptor groups are produced in excess, and not only replace those combined by the toxins, but the excessive groups escape free into the blood. Hence the serum of an immunized animal is antitoxic *because it contains free cell receptors* that can unite with the toxin. An important point is that the receptors liberated by all animals which have been immunized with a given toxin seem to be the same—horse serum, or sheep serum, or goat serum will neutralize diphtheria toxin if the animals have been made immune to this toxin; and, furthermore, their serum when introduced into the body of an entirely different animal, *e. g.*, a guinea-pig, will neutralize diphtheria toxin within its body. Equally important is the fact that the antitoxin for one toxin will not neutralize any other toxin; *e. g.*, diphtheria antitoxin will not neutralize

tetanus toxin, or conversely. This means that diphtheria toxin is attached to chemical groups of the body cells (receptors) which are quite different from the groups to which tetanus toxin unites, and hence different receptors are thrown out in immunizing against each.

The neutralization of toxin by antitoxin is distinctly a chemical process, which occurs as well in the test-tube as in the body. It occurs *according to the laws of definite proportion*, a given amount of antitoxin neutralizing a proportionate amount of toxin under equal conditions (hence the toxin is not destroyed by antitoxin through a ferment action, as was at first suggested). Neither the toxin nor the antitoxin is destroyed in the process of neutralization, as has been proved by suitable experiments, but they appear to be chemically united to each other, as any two large molecules may be. Neutralization occurs more rapidly under the influence of warmth, and more slowly in the cold; and it is more rapid in concentrated than in dilute solutions, just as with ordinary chemical reactions. According to Arrhenius and Madsen, the reaction of antitoxin upon toxin is accompanied by the liberation of much heat—6600 cal. per gram molecule, or about half as much as is set free by the action of a strong acid upon a strong base.¹ On dilution of a neutral toxin-antitoxin mixture, a certain amount of dissociation seems to occur.²

There is no relation between antitoxins and enzymes. The antitoxin acts quantitatively, and produces no detectable alteration in the toxin, or in any other substance, as far as we know. It also has but one functioning group (haptophore), the one with which it combines with the toxin; whereas both toxins and enzymes seem to have two functioning groups, one which unites with the cell or substance that is to be attacked, the other which produces the chemical changes.

CHEMICAL NATURE OF ANTITOXINS

This is as entirely unknown as is the nature of the toxins. Investigation of antitoxic serum (principally diphtheria antitoxin) has shown that the antitoxic properties are closely related to the serum globulin, which, however, by no means proves that antitoxin is serum globulin or any other sort of a proteid. According to Ehrlich's theory, antitoxin consists of free cell receptors, and these receptors are presumably simple chemical

¹ Literature of chemical and physical reactions of toxin and antitoxin given by Zanger, *Cent. f. Bakt. (ref.)*, 1905 (36), 238.

² See Otto and Sachs, *Zeit. exp. Path. u. Ther.*, 1906 (3), 19.

groups which may be but a part of a larger molecule, or they may be entire proteid molecules. In any event they behave as colloids¹; moving toward the anode in an electrical field, diffusing little or not at all, their reaction curve resembling more an absorption curve than the reaction curves of crystalloids, and being influenced by all conditions that influence colloids. Whether the receptor groups are secreted in a free condition in antitoxin formation or combined in a large molecule is unknown.

By saturating serum with magnesium sulphate, or half saturation with ammonium sulphate, three chief groups of proteids can be precipitated and isolated.² These are *fibrinogen*, *euglobulin* (true globulin), and *pseudo-globulin* (soluble in water). Belfanti and Carbone³ found that diphtheria antitoxin was carried down in the globulins obtained by salting out with ammonium or magnesium sulphates, but not in the precipitates obtained with acetic acid. Atkinson⁴ found that the globulin thrown down on saturating serum with magnesium sulphate contained all the antitoxin. Reprecipitating this globulin with NaCl at different temperatures in five different fractions, each fraction was found to contain a part of the antitoxin, the five fractions together containing the entire antitoxic strength.

Glaessner⁵ could not find any perceptible increase in the amount of globulin in the serum after immunization. Pick⁶ found that the precipitate obtained by 36 per cent. volume saturation with ammonium sulphate contained no antitoxin; the antitoxin came down in the precipitate obtained on raising the strength from above 38 per cent. to 46 per cent. According to Pick, in horse serum the antitoxin is associated with the pseudo-globulin. He gives the following table of distribution of different immune bodies in the serum of different animals:

Immune body.	Fibrinoglobulin.	Euglobulin.	Pseudo-globulin.	Albumin.
Diphtheria antitoxin . . .	0	Goat	Horse	0
Tetanus antitoxin . . .	0	Goat milk (?)	"	0
Cholera hemolysin . . .	0	Goat	0	0
Typhoid agglutinin . . .	0	{ Goat, rabbit, Guinea-pig	Horse	0
Cholera agglutinin . . .	0	Horse, goat	0	0

¹ See Zangger (*loc. cit.*).

² See résumé by Gibson, *Jour. Biol. Chem.*, 1905 (1), 161. Literature in "Toxine und Antitoxine," Oppenheimer, 1904, p. 81.

³ *Cent. f. Bakt.*, 1898 (23), 906.

⁴ *Jour. Exper. Med.*, 1901 (5), 67.

⁵ *Zeit. f. exp. Path. u. Ther.*, 1905 (2), 154.

⁶ Hofmeister's Beitr., 1901 (1), 351.

Atkinson¹ attempted to determine the proteid nature of antitoxin by the biological method (*i. e.*, by means of the precipitin reaction). He immunized rabbits with globulin obtained from normal horse serum and with globulin from antitoxic serum, and in either case obtained a serum precipitating the globulin of antitoxic serum, and with it all the antitoxin. This experiment merely shows that the antitoxin is carried down with the globulin precipitates and does not prove that it is itself a globulin. When the precipitating serum was added to a neutral mixture of toxin and antitoxin, it did not separate the antitoxin from the toxin and leave the latter free, indicating that the toxin-antitoxin union is quite firm.

The relation of antitoxins to proteids has also been investigated by permitting digestive enzymes to act on antitoxic serum. Pick² digested the antitoxin-containing globulin of horse serum for several days with trypsin; after five days, when part of the albumin was still not digested, the antitoxin was but little impaired in strength; after nine days, when most of the proteid was digested, the antitoxin had lost two-thirds of its strength. This indicates a considerable resistance of antitoxin to trypsin, but also shows that it is affected in much the same way as the globulin (which is itself very resistant to trypsin) and therefore is presumably of similar nature. Antitoxin seemed to be much more rapidly destroyed by pepsin-HCl digestion than by trypsin, in which respect it again resembles the serum globulin.

In favor of the view that antitoxin is a definite proteid body is also the fact that it is not carried down in indifferent precipitates, as are the enzymes, but comes down always in a certain fraction of the proteid precipitates, *e. g.*, we can precipitate all the serum albumin from an antitoxic serum, and it does not carry down with it any of the antitoxin. Another important point has been brought out by Arrhenius and Madsen,³ who determined approximately the molecular weight of toxin and antitoxin by means of their rate of diffusion, and found that the toxin (diphtheria toxin and tetanolysin) diffused ten or more times as rapidly as the corresponding antitoxin. This indicates that the antitoxin molecules are much larger than the toxin molecules, agreeing with the idea that antitoxin is of proteid nature and that toxin is not.

Taken all together, the evidence indicates a closer resemblance of antitoxins to proteids than has been shown for the toxins,

¹ Medical News, 1904 (84), 375.

² *Loc. cit.*

³ Festskrift Statens Serum Institut, 1902.

and all attempts to separate antitoxins from proteids have so far failed.¹

Antitoxins are retained to greater or less extent by porcelain filters, do not pass through dialyzing membranes readily, and are in general easily destroyed by chemical and physical agencies, although much less so than are most toxins. Heating to 60°–70° injures, and boiling quickly destroys them, although like the enzymes and the proteids, they resist dry heat to 140°, and also extremely low temperature, without change. Putrefaction of the serum destroys the antitoxins (Brieger²). They can be preserved for a very long time when dried completely, but in the serum they gradually disappear, especially if exposed to light and air. Acids and alkalies destroy antitoxins, acids being the more harmful in low concentrations. They are destroyed in the alimentary tract, without appreciable absorption, except in the case of new-born animals suckling mothers whose blood and milk contain antitoxin (Römer and Much³). When subcutaneously injected, antitoxin soon disappears from the blood; part may be bound to the tissues, part may be destroyed, since only traces appear in the urine.

Toxicity of Serum.—Antitoxin itself seems to be quite free from poisonous effects. The intoxications observed after injections of antitoxic serum *are not due to the antitoxin, but to the serum itself*. Foreign serums, as well as proteids of all kinds, sometimes exert a markedly poisonous influence upon animals into whose circulation they have been introduced. This is manifested not only by sickness and anatomical lesions, but also by the production of specific precipitating bodies in the blood (see “Precipitins”). But if we inject antitoxic serum (for diphtheria) derived from horse blood into another horse, it is quite without toxic effect.

An interesting phenomenon has been observed in the immunization of animals, namely, that whereas a small dose of a foreign serum may be borne without serious effects, a repetition of the injection after an interval of ten days or more is followed by profound and often rapidly fatal intoxication (this has been called the Theobald Smith phenomenon). The first dose of serum makes the animal susceptible to even a small dose of the same serum (and somewhat susceptible to other serums) which seem to act on the respiratory center. As small a quantity of

¹ An exception is claimed by Pröscher (Münch. med. Wochenschr., 1902 (49), 1176), which Brieger could not substantiate (Festschrift f. Koch, 1903, p. 445). Römer (quoted by v. Behring, Beitr. z. exp. Therap., 1905, Heft 10, p. 22) found that tetanus antitoxin will partly escape through a dialyzing membrane, and in the antitoxin-containing dialysate no proteid can be found by ordinary precipitation reactions; but by ultra-microscopic methods proteids can be found in every antitoxin-containing dialysate.

² Behring states that tetanus antitoxin resists putrefaction.

³ Jahrb. f. Kinderheilk., 1906 (13), 684.

horse serum as from 0.004 to 0.000001 c.c. was found sufficient to render a guinea-pig susceptible, and 0.1 c.c. was sufficient to kill a guinea-pig that had been thus sensitized. Possibly this fact of development of susceptibility may play an important part in the cases of intoxication following administration of antitoxic serum.¹

IMMUNITY AGAINST BACTERIAL CELLS

By far the greater number of pathogenic bacteria do not produce true soluble toxins, but form toxic materials which accumulate within the cells, *endotoxins*; these produce intoxication only when the bacterial cells are disorganized, liberating the endotoxins. Against such endotoxins no antitoxic substances have yet been produced by immunization.² The same is true of the non-specific bacterial proteids. A certain degree of immunity can be conferred to animals against the poisonous proteids isolated from various bacteria in Vaughan's laboratory,³ but it is not comparable in any way to antitoxic immunity. Hence these endocellular poisons are in some way different from the true soluble toxins.

If we immunize an animal against living bacteria, or against the dead bodies of bacteria, or against endotoxins, and examine the properties of its serum, we find that although the serum is powerless to neutralize the poisonous effects of the bacterial constituents, it does possess other marked properties, which are quite the same no matter which of the materials mentioned was used in immunization. The serum will kill bacteria both in the test-tube and in the animal body; that is, it is *bactericidal*. It contains substances that cause the bacteria to agglutinate, called *agglutinins*; and if motile, to lose their motility. It contains substances that render the bacteria more readily ingested by phagocytes; these substances are called *opsonins*. And also this serum will inhibit the action of the bacterial enzymes, and will produce a precipitate in solution of the bacterial proteids, *i. e.*, it contains *antienzymes* and *precipitins*. All these properties are, to a certain extent, specific; that is, they are exerted chiefly or solely against the particular form of organism that was used in immunizing.⁴ Each property is also quite

¹ Full discussion by Rosenau and Anderson, Bull. No. 29, U. S. Gov't Hygienic Lab., 1906; Jour. Med. Research, 1906 (15), 179. Also see Wolff-Eisner, Cent. f. Bakt., 1906 (40), 634; and Otto in v. Lenthold's Gedenkschr., 1906 (1), 1.

² Besredka (Ann. Inst. Pasteur, 1906 (20), 149) and a few others claim to have secured antiendotoxins.

³ American Med., 1905 (10), 145.

⁴ Welch (Med. News, 1902 (81), 721) has suggested that possibly the bacteria in their turn may develop antibodies for the tissues and fluids in which they are growing. If so, we have a reasonable explanation of the development

distinct from the others and may therefore be considered by itself.

BACTERICIDAL SERUM

The bactericidal property of serum may be shown by its destruction of the life manifestations of bacteria without marked alteration in their structure, or it may be accompanied by dissolution of the bacterial cell (*bacteriolysis*). How much of the bacteriolytic process is performed by the serum itself, or how much by the autolytic enzymes of the bacterial cell, is unknown, but the latter is probably an important factor. The bactericidal property of immune serum has been shown to be quite independent of the antitoxic properties and also to have quite a different mechanism. This last is shown in the following manner:

If we heat bactericidal serum made by immunizing an animal against bacteria, say the cholera vibrio, at 55° for fifteen minutes, it will be found to have lost its power of destroying these organisms. Normal serum of non-immunized animals is equally without effect upon the vibrios. If, however, we add to the inactive heated serum an equal quantity of inactive normal serum, the mixture will be found to be as actively bactericidal as the original unheated immune serum. This phenomenon is interpreted to mean that, by immunization, some new substance has been developed which, although by itself incapable of destroying bacteria, is able, when united with some substance present in normal serum, to destroy bacteria readily. The substance present in normal serum is also incapable of affecting bacteria by itself, but needs the presence of the substance developed by immunizing to render it bactericidal. Hence *the bactericidal property in this case depends on two substances acting together*: one, developed during immunization and therefore called the *immune body*, is specific for the variety of bacteria used in immunization, and is not destroyed by heating at 55° . The other, present in normal serum, is not increased during immunization, is not (altogether) specific in character, and is destroyed by heating at 55° ; as its action is complementary to that of the specific immune body, it is called the *complement*.

It is believed that the action of these substances is as follows: The immune body is, like antitoxin, a cell receptor which unites

by bacteria of marked selective action for specific cells of the host; *e. g.*, leucolysins, endotheliolysins, hemolysins, etc.; and also the peculiar manner in which bacteria often attack only certain tissues, *e. g.*, multiple septic arthritis. The fact that bacteria are said to develop enzymes with specific effects according to the media upon which they grow is in support of this hypothesis.

the bacteria or their poisonous constituents to the cell. It differs from the antitoxin, however, in that it has two affinities, one for the complement and the other for the bacterial substance. On account of the existence of the two affinities it is called an *amboceptor*. Some serums contain such amboceptors for certain bacteria without previous immunization, hence the term *immune body* is reserved for amboceptors developed by immunization.

Amboceptor and Complement.—The function of the amboceptor is to unite the bacterial protoplasm, to which it is attached by one affinity, to the complement which it holds by its other affinity, or, to put it in a more strictly chemical way, the addition of the amboceptors to the bacteria gives them a chemical affinity for complement. It is, therefore, an *intermediary body*, uniting the complement to the bacterial protoplasm. The complement is the substance that actually destroys the bacteria, in which respect, as well as in its susceptibility to heat, it resembles the enzymes. Complement is present in normal serums, and, as it is not increased in amount during immunization, it may not be sufficient to satisfy all the amboceptors, hence it may be impossible to secure marked bactericidal effects even when many amboceptors have been formed. If the complement in an immune serum has been destroyed by heating, it may be replaced by adding normal serum from another animal, even of some other species; indicating either that the complement is not absolutely specific in its nature, or that quite the same complement may be present in the blood of many different animals. The origin of the complement is unknown, but it has been urged that the leucocytes are an important source of this substance, if not its chief one; there is evidence, however, that various organs and cells may also produce complement. Its most prominent characteristics are its extreme susceptibility to heat, and the resemblance of its action to the action of enzymes.¹ Hektoen² found that it could be made to unite with Mg, Ca, Ba, Sr, and SO₄ ions, which rendered the complement (for typhoid bacilli and red corpuscles) inactive. Manwaring³ found that these ions could be separated again from the complement by simple chemical precipitation.

According to the Ehrlich theory, complement, like toxins and enzymes, possesses at least two groups: one, the haptophore, with which it unites with the amboceptor; the other, the toxophore (or *zymophore*, because of its enzyme-like action), which

¹ See Walker, Jour. of Physiol., 1906 (33), p. **xxi**.

² Trans. Chicago Path. Soc., 1903 (5), 303.

³ Jour. Infectious Diseases, 1904 (1), 112.

attacks the bacterial protoplasm. It may degenerate and lose its toxophore group while retaining the power to combine by means of its haptophore group, thus forming a *complementoid*. Complement and amboceptor exist side by side in the serum, not uniting with one another until the amboceptor has become attached to the bacterial protoplasm.

In its effect of dissolving bacteria (and also other cells against which animals may have been immunized) *complement resembles the enzymes*, and it is generally looked upon as related to them.¹ As yet, however, none of the products of proteolysis has been isolated from substances acted upon by complement, nor do the changes it produces resemble those produced by proteolytic enzymes in all details. In particular, complement seems to participate in reactions according to the law of definite proportions, unlike the enzymes.² The chemical nature of complement seems to be entirely unknown. In certain immune reactions, colloids (lecithin, silicic acid³) can play the rôle of complement and immune body, but these reactions are probably quite different from those of bacteriolysis by immune serum.

Immune body (amboceptor) is formed, according to Wassermann, and Pfeiffer and Marx, in the spleen and hemopoietic organs, since in immunization it can be demonstrated in these organs before it appears in the circulating blood. The resistance of immune bodies is very considerable: serum prepared in 1895 by Pfeiffer against cholera vibrios was found to have lost almost none of its activity after eight years in an ice-box (Friedberger). Heating twenty hours at 60° scarcely injures them, but 70° for one hour destroys them almost completely, and heating the serum to 100° destroys all the immune bodies. They are quite resistant to putrefaction, and, like the antitoxins, do not dialyze.

According to Pfeiffer and Proskauer,⁴ digestion of the globulin precipitate, in which immune bodies are carried down, does not destroy their activity completely even when all the proteids are thus removed. Removal of the nucleo-albumin or

¹ The suggestion has been made that bacteriolysis, even in immune serum, depends upon osmotic disturbances. Looz and Tallant (Johns Hopkins Hosp. Bull., 1900 (11), 220) tested the electrical conductivity of the serum before and after heating to 57°, and found no change, speaking strongly against this rather poorly based hypothesis. Leuchs (Arch. f. Hyg., 1905 (54), 396) also failed to find evidence that bacteriolysis by immune serum is due to osmotic changes. As regards the resemblance of bacteriolysis to proteolysis, see Turró, Berl. klin. Woch., 1903 (40), 821.

² See Liebermann, Deut. med. Woch., 1906 (32), 249.

³ Landsteiner and Jagic, Wien. klin. Woch., 1904 (17), 63; Münch. med. Woch., 1904 (51), 1185.

⁴ Cent. f. Bakt., 1896 (19), 191.

nuclein does not remove the immune bodies from the serum. Immune serum kept three months in alcohol yielded an extract with distilled water that was rich in immune bodies, but almost free from proteid. Pick, Rhodain, and Fuhrmann found that immune bodies are precipitated entirely in the euglobulin fraction of the serum proteids. From these experiments it seems probable that the immune body is not itself a proteid, although closely associated with the serum globulins.¹

Opsonin.²—Bactericidal substances are not so readily produced for all bacterial organisms as they are for typhoid bacilli, cholera spirilla, etc., particularly not for the pus cocci, *B. anthracis*, and *B. tuberculosis*. In defending the body against these organisms it would seem that phagocytosis by leucocytes is an important process, but in just what the difference lies between immunized and normal animals was formerly not clear. It now seems to have been established, particularly by the work of Wright and Douglas,³ that phagocytosis depends upon the presence of certain substances in the plasma, which they call *opsonins*. Not until bacteria have been acted upon by the opsonin can they be taken up by the phagocytes. Opsonin exists in the normal blood of many animals, and can be increased by immunization, and the opsonin of one species of animal can sensitize bacteria for the phagocytes of another species. It resembles toxin and complement in having a haptophore group to combine with the bacteria, and an opsoniferous group susceptible to heat of 60°–65°; when thus heated, the opsonin is converted into an *opsonoid*. Nothing is yet known concerning the change brought about in the bacteria by the opsonin, although it has been established that it is the bacteria that are modified and not the leucocytes. The chemical nature of the opsonins is likewise unknown, except that they may combine with certain inorganic ions and are then inert (Hektoen and Ruediger⁴). This topic is discussed further in connection with phagocytosis.

Antienzymes.—The development of substances inhibiting the action of bacterial enzymes during the course of immunization has been discussed in a preceding chapter (under “Enzymes”). Their importance

¹ Ascoli found that the active substance of anthracidal serum, which is not an amboceptor, is contained in the pseudo-globulin fraction of asses' serum, but in goat's serum part is in the euglobulin fraction. (Biochem. Centr., 1906 (5), 458.)

² Résumé and literature by Hektoen, Jour. Amer. Med. Assoc., 1906 (46), 1407.

³ Proc. of the Royal Society, 1903 (72), 357; 1904 (73), 128.

⁴ Jour. Infectious Diseases, 1905 (2), 129.

in defense against infection is, however, questionable, as we have no evidence that the bacterial enzymes cause harm to the infected organism, or that the products of their action are particularly toxic. By preventing the assimilation of food by the bacteria, however, antienzymes might inhibit bacterial growth, a possibility that seems not to have been investigated.

AGGLUTININS AND AGGLUTINATION

This well-known phenomenon, the clumping or agglutination of bacteria when acted upon by the serum of immunized or infected animals, can hardly be considered as a means of defense, since we have no evidence that it in any way protects the animal. Agglutinated bacteria seem not to be severely injured by the process, and can grow vigorously in agglutinative serum. Possibly agglutination favors phagocytosis and lessens dissemination of the infecting organisms, but it is improbable that the influence on the course of infection is great. Agglutination, therefore, may be looked upon as an incident in the infection, rather than as a definite method of resistance.

For the production of agglutination it is necessary that the bacterial body contain a substance (*agglutigen*) which has an affinity for the specific constituent of the serum, *agglutinin*. Normal serum may contain agglutinin; *e. g.*, typhoid bacilli are sometimes agglutinated by normal serum, even when it is diluted thirty times, but by immunization this property can be greatly increased until agglutination may be obtained with dilutions as high as one to a million. In immunization it is believed that the agglutigen, which is probably an intracellular constituent of the bacteria, acts as a stimulator to the formation of the specific agglutinin. Hence, when we inject extracts of cells containing endotoxins, we secure agglutinins, for the agglutinogens are liberated from the cells under the same conditions as the endotoxins.

We can obtain agglutinins against nearly all bacteria, including non-pathogenic forms, but in varying strengths. Agglutinins are found in the blood stream in the highest concentration, but they are also present in the various organs and in the milk. The place of their formation is unknown. Since bacteria contained within a collodion sac implanted in an animal give rise to the production of agglutinins, it is evident that the agglutinogens are diffusible to some extent, at least, through collodion. Old cultures of bacteria contain free agglutinogens, probably liberated from disintegrated cells, and filtrates of such cultures will neutralize agglutinins, showing both that the agglutinogens are filterable, and that the reaction of agglutination is a chemical

one and not dependent upon the presence of cells. Agglutinogens are said to pass through dialyzing membranes, while agglutinins do not.

Properties of Agglutinins.—Like most of the other substances produced in immunity, agglutinins are precipitated out of the serum in the globulin fraction (see Pick's table, p. 140). All attempts to separate them from proteids have been unsuccessful. Stark¹ found that trypsin does not attack the agglutinins readily, corresponding to the resistance of the serum proteids to this enzyme; alkaline papayotin solution destroys them slowly, while pepsin acts much more rapidly. Alkalies are destructive even when quite dilute, while acids are much less harmful. The temperature resistance of agglutinins seems to be variable, plague agglutinin being destroyed at 56°, while purified typhoid agglutinin may resist 80°–90°; most agglutinin serums lose their property at 60°–65°. The rate of reaction of agglutinins increases with the temperature, as long as this is not high enough to injure the reacting substances.²

The structure of the agglutinins (in the Ehrlich theory) is similar to that of the toxin; *i. e.*, there is a haptophore group by which they combine with the agglutininogen, and a toxophore group by which they produce the changes that cause agglutination. The agglutininogen is probably related to the antitoxins in structure, having a single haptophore to unite with the agglutinin. By degeneration of the toxophorous group of the agglutinin, *agglutinoids* may be formed. It is believed that agglutinins are cell receptors, which have a group with a chemical affinity for the agglutininogen of the bacterial protoplasm, and also another group which brings about the agglutination. They are, therefore, more complex than the simple receptors that unite with toxins, and are called *receptors of the second order*.

Just what constituent of the bacteria acts as the stimulus to the production of the agglutinin is unknown. Apparently, there are at least two bacterial substances with this property, one of which seems not to be a proteid, since it is soluble in alcohol and gives no biuret reaction, and resists temperature up to 165°. The other gives all proteid reactions, and is destroyed by heating to 62°. We consider, therefore, that there are two agglutinogens in the bacterial cell, one, thermostable, the other, thermolabile. The difference in the function of these two

¹ Inaug. Dissert., Würzburg, 1905.

² Madsen, *et al.*, Jour. Exper. Med., 1906 (8), 337.

agglutinogens is still a matter of dispute.¹ Likewise, the question as to whether they occur in the membrane or within the bacterial cell is still open, but Craw² found that the insoluble residue of crushed typhoid bacilli, after being washed free of all soluble constituents, was but slightly agglutinated by active serum; therefore, the agglutinogens are probably soluble intracellular substances.

Agglutinated bacteria can be again separated from one another by the action of organic and inorganic acids, alkalies, acid salts, and by heating to 70° or 75°, and after once being separated they cannot be reagglutinated by fresh serum.³

The Mechanism of Agglutination.—This has been a fruitful field of research, in which the application of physical chemistry has been very profitable. At first it was believed that the clumping was brought about by loss of motility, until it was found that non-motile bacilli were equally affected. Similarly, the hypothesis of adhesion of the flagellæ was disposed of. Gruber⁴ and others supposed that a sticky substance, "*glabrificin*," was absorbed from the serum by the bacilli, which caused them to adhere on contact with one another; but this does not explain the flocking together of non-motile bacilli. Paltauf considered that the specific precipitin (see next section) produced by immunization carried the bacilli down in the precipitate formed, and there is reason to believe that this reaction is of importance, but it does not explain all the facts of agglutination, nor is the relation between agglutinating and precipitating power of immune serums a constant one. Neisser and Friedmann⁵ found that if the bacterial cells were saturated with lead acetate, washed in water until all soluble lead was removed, and then treated with H₂S, they were promptly agglutinated and precipitated, supporting other observations that indicate that precipitation within the bacterial cells can lead to agglutination. This sort of agglutination is probably related to the process of formation of coarse flocculi in solutions, and probably depends upon alterations in surface tension.

Bordet⁶ made the important observation that agglutination would not occur if both the bacterial suspension and the agglutinating serum were dialyzed free from salts before mixing; but if, to such mixtures, a small amount of NaCl was added, agglu-

¹ See Paltauf, Kolle and Wassermann's Handbuch, Bd. 4, p. 726.

² *Loc. cit.*, *infra*.

³ Eisenberg and Volk, Zeit. f. Infektionskr., 1902 (40), 192.

⁴ For complete bibliography, see Craw, Jour. of Hygiene, 1905 (5), 113.

⁵ Münch. med. Woch., 1904 (51), 465 and 827.

⁶ Ann. d. l' Inst. Pasteur, 1899 (13), 225.

tion and precipitation of the bacteria occurred at once. This observation brought the phenomenon of bacterial agglutination into close relation with the precipitation of colloids by electrolytes, Bordet comparing it to the precipitation of particles of inorganic matter suspended in the fresh water of rivers that occurs when the fresh water meets the salt water of the ocean. He found that the agglutinin combined with the bacteria in the absence of the salts, and the resulting compound was precipitated by the addition of minute amounts of electrolytes, which did not precipitate or agglutinate the bacteria or the serum alone. This indicates that the agglutinins cause a change in the bacteria which brings them under the same physical laws as the inorganic colloidal suspensions, which are characterized by being precipitated by the addition of traces of electrolytes.¹ This precipitation is undoubtedly due to changes in solution tension and surface tension (see "Precipitation of Colloids," introductory chapter). Before the agglutinin combines with the bacteria they behave like the colloidal solutions of organic colloids, being only precipitated by the salts of heavy metals, alcohol, formalin, etc., or by great concentrations of neutral salts.

According to Bechhold² normal bacteria behave like inorganic suspensions that have each particle protected by an albumin-like membrane, which prevents them from being thrown out of suspension by solutions of alkali salts, etc. After being acted on by agglutinin they are so altered that they behave like the unprotected inorganic suspensions, and are precipitated by salts and other electrolytes. This suggests the possibility that the agglutinin makes the bacteria permeable for these electrolytes. Agglutination obeys the same laws as other similar physical phenomena; the rate of agglutination depends upon the concentration of the suspension and of the electrolytes, and varies with the valence of the cations. Although bacteria in an electric stream move toward the anode like all suspensions, after being acted on by agglutinin they are agglutinated by the current between the poles; this indicates the importance of the electrical charges of the bacterial surfaces in their agglutination reactions.

In all respects the behavior of bacteria and agglutinin resembles the behavior of colloidal mixtures in suspension

¹ Arrhenius (*Zeit. physikal. Chem.*, 1903 (46), 415) has attempted to show that the gas laws are applicable to the partition of agglutinin between the bacteria and the medium, which he compares to the partition of iodine between water and carbon disulphide. This idea is not accepted by Craw (*loc. cit.*).

² *Zeit. f. physikal. Chem.*, 1904 (48), 385.

(Neisser and Friedmann¹) which form an electrically amphoteric colloidal suspension, so that the ions of electrolytes or the electric currents, by discharging them unequally, cause precipitation. Physico-chemical researches, however, have yet failed to explain the specific character of the agglutinins for specific bacteria.

PRECIPITINS²

If to the filtrate from a bacterial culture we add in proper proportions the serum of an animal immunized against the same variety of bacteria, or against their cell contents, a precipitate will soon form. This reaction is specific in that it is produced to a much less degree, or not at all, with cultures of bacteria different from the variety used in immunization. The precipitated substances seem to consist of the soluble proteid constituents of the filtrate derived from the bacterial cells.

This reaction seems to be of little significance as a means of defense against bacterial invasion; but the discovery that all forms of proteids when injected into animals may cause the appearance of specific precipitating substances in the serum of the animals, has led to most important applications of the precipitation reaction. Apparently every variety of proteid is in a certain sense poisonous to animals that do not normally have it in their blood or tissues, and its injection leads to a reaction on the part of the animal, which reaction is shown by symptoms of sickness and the appearance of the specific precipitating substances in the serum. It is the sharp limits of specificity that render the precipitin reaction of such importance, for if we immunize an animal with globulin from the blood of a horse, its serum will precipitate only globulin from horse blood, and not globulin from the blood of a dog, or man, or any other animal. Similarly, if the immunization is with cow's milk, the serum will precipitate only cow's milk and not the milk of the goat or mare. These serum reactions are of importance to the physiological chemist, therefore, since they furnish a means of distinguishing between closely related forms of proteids, more delicate by far than any known chemical reagent. They also prove that there are essential chemical differences between the proteids of

¹ *Loc. cit.*; see also Girard-Mangin and Henri, *Compt. Rend. Soc. Biol.*, 1904, vol. 56; and Zangger, *Cent. f. Bakt. (ref.)*, 1905 (36), 225.

² For complete bibliography of the subject of "Precipitins" see the résumé by Michaelis, *Biochemisches Centralblatt*, 1905 (3), 693; and *Zeit. f. klin. Med.*, 1905 (56), 409; Blum, *Cent. allg. Path.*, 1906 (17), 81; Pfeiffer, *Arch. f. Kriminalanthropol.*, 1906, Bd. 22. For methods and earlier literature see Nuttall, *Jour. of Hygiene*, 1901 (1), 367.

different species of animals, even when by all other methods these proteids seem to be practically identical; *e. g.*, lactalbumin of cow's milk is in some respect different from lactalbumin of goat's milk since it produces a different precipitin. To the physiologist they indicate the method adopted by the body to guard itself against invasion by foreign proteids introduced in the food, and show the importance of the complicated digestive and assimilative mechanism of the alimentary tract in securing complete destruction of the specific characters of all proteid foods before they enter the blood. Clinically they offer a means of detecting abnormal permeability of the walls of the digestive tract, and possibly a means of determining the source of proteids found in the urine. Medicolegally they offer an accurate method of determining the origin of blood and serum stains, no matter how old the stain may be; thus Hansemann¹ found that material obtained from a mummy 5000 years old gave the precipitin reaction.

Production of Precipitins.—For the production of the precipitation reaction it is necessary to have in the substance used for immunization a certain group, the *precipitogen*, which, when injected gives rise to production of *precipitin* by the animal.² Apparently any proteid may act as a precipitogen if injected into the proper animal, but it *must be a foreign proteid*; rabbit serum will not produce precipitins if injected into a rabbit,³ probably because it is normally present in the blood of the rabbit and therefore does not stimulate any reaction. In general the more foreign the proteid, the greater the amount of precipitin; closely related animals, *e. g.*, rabbit and guinea-pig, produce little precipitin for one another's proteids. This indicates distinctly that difference in species depends upon or is associated with difference in chemical composition of the proteids. Only proteids can produce precipitins; when split to the peptone stage they lose this property. No precipitins can be secured against the other food-stuffs; *i. e.*, carbohydrates and fats. Possibly precipitins can be produced for closely related substances with molecules approximating in size the proteid molecule, *e. g.*, certain substances present in proteid-free filtrates of bacterial cultures.

Since precipitation of colloids is accompanied by or dependent

¹ Münch. med. Woch., 1904 (30), 572.

² Kraus and Schiffmann (Wien. klin. Woch., 1905 (18), 1033) believe that precipitins as well as agglutinins are formed in the circulating blood, not in the organs.

³ Rarely a slight reaction against homologous proteids has been obtained (*iso-precipitins*).

upon an aggregation of their particles, the precipitin reaction is closely related to the agglutination reaction. The amount of precipitation obtained is much modified by the amount of inorganic salts present, and, according to Friedmann,¹ there is a general resemblance between the precipitin reactions and the precipitations occurring when colloids precipitate one another; *i. e.*, when an amphoteric colloid reacts with either an acid or a basic colloid. Possibly the constituents of the nucleoproteids furnish the acid and basic colloids for these reactions.² As mentioned in the preceding section, agglutination of bacteria is independent of the precipitins, although very probably influenced by them. As with all the other substances of this class, the precipitins have a haptophore group by which they unite to the proteid molecule, and another group by which they produce the change resulting in precipitation. When the latter group is destroyed by heating to 60°, the precipitin is converted into a *precipitoid*.

According to the source of the proteid used we recognize *bacterial precipitins*, *phyto-precipitins* (for plant proteids), and *zoöprecipitins* (for animal proteids). Although tissue extracts, body fluids, and exudates are generally used in immunizing, purified constituents of these proteid mixtures will also excite precipitin formation, *e. g.*, we may immunize with caseinogen as well as with milk. Immunization with frequently recrystallized albumins is less successful (Obermayer and Pick). Complete pepsin digestion of proteids deprives them both of their precipitability and their power to produce precipitins, the former property being lost first. Trypsin seems to produce the same effect more slowly. Heating to coagulation—indeed, heating in the autoclave—does not destroy the precipitogenous property of proteids, but modifies somewhat the character of the precipitin obtained.³

As proteids introduced into the stomach are normally destroyed before being absorbed, they do not enter the blood and cause precipitin formation. However, as is well known, eating of excessive amounts of egg-albumen or other easily absorbed proteids may result in their passing the barriers and entering the blood stream, and in this way precipitins have been experi-

¹ Arch. f. Hyg., 1906 (55), 361.

² See Friedmann and Friedenthal, Zeit. exp. Path. u. Ther., 1906, (3) 73.

³ See Obermayer and Pick, who consider in detail the effects of various modifications of proteids upon their power to incite precipitin formation (Wien. klin. Woch., 1906 (19), 327). The precipitability of the serum, or its power to produce precipitins, is not affected by disease (Pribram, Zeit. exp. Path. u. Ther., 1906 (3), 28).

mentally produced.¹ Presumably the precipitin reaction is a means of throwing such foreign proteids out of solution and rendering them harmless.

Precipitin appears in the blood generally about six days after injection of the proteid, but disappears after injection of each subsequent dose of proteid, to reappear again after a somewhat shorter lapse of time. After injections are stopped, the precipitin disappears rather rapidly, but never appears in the urine, although it may enter the fetal blood from the blood of pregnant female animals. Leucocytosis, both local and general, follows the serum injection, and it has been suggested that the leucocytes are the source of the precipitin. The presence of precipitins in the blood does not seem to prevent the excretion of the foreign proteid in the urine, nor are the animals less susceptible to the toxic action of the foreign proteid; indeed, the reaction is even stronger in the immunized animals, and sometimes the ordinary dose becomes fatal,² as mentioned previously under "Toxicity of Serum."

Chemical Properties.—In its *chemical nature* precipitin resembles the "antibodies" generally, being precipitated in the euglobulin fraction of the serum,³ and slowly destroyed by trypsin, rapidly by pepsin. It cannot be separated from the serum proteids.

Specificity.—As regards the *specificity of precipitin reactions* certain points must be considered. Precipitin against human albumin reacts with human globulin, but not with either horse albumin or globulin. The groups that react, therefore, are characteristic of the species, but common to different proteids of the same species. This group does not occur in all proteids, however, even in the same species, for precipitins against cow's serum do not react with cow's milk. Bacterial precipitins react frequently with members of an entire group. For example, serum of animals immunized against *B. typhosus* may produce precipitates in filtrates from cultures of numerous other members of the colon-typhoid group, although quantitative differences exist in favor of the form used in immunizing.⁴ Likewise precipitins for the serum of one animal will produce

¹Concerning the toxicity of egg-albumen, see Sollmann and Brown, Jour. Exp. Med., 1902 (6), 207.

²See Rosenau and Anderson, U. S. Gov't. Dept. of Hygiene Bull., No. 29, 1906; Jour. Med. Research, 1906 (15), 179.

³Funck (Cent. f. Bakt. (ref.), 1905 (36), 744) states that if the precipitin serum is very strong, part of the precipitin comes down in the pseudoglobulin.

⁴For literature on Bacterial Precipitins see Norris, Jour. of Infectious Diseases, 1904 (1), 463.

precipitates in the blood of related animals; *e. g.*, immune serum against human serum will cause precipitates in the serum of the higher apes. The precipitin reaction is, therefore, only quantitatively specific, not qualitatively.

The precipitin reaction occurs only outside the body (Michaelis). When serum precipitins are injected directly into the blood, no precipitation occurs, but merely an active leucocytosis; if injected intraperitoneally, there is local leucocytosis. Probably the leucocytes take up the precipitate as fast as formed, or else the absence of precipitate depends upon the fact that a proper proportion between the amount of precipitin and proteid must exist, an excess of proteid causing a resolution of the precipitate.

The precipitation by precipitins is not an enzyme action, for the precipitins are used up in the process. It apparently does not differ from precipitations of colloids by other colloids of opposite electrical charges, except in that the reaction is specific.

CHAPTER VII

CHEMICAL MEANS OF DEFENSE AGAINST POISONS OF KNOWN COMPOSITION

ALTHOUGH the examples of acquired immunity against poisons of known chemical composition are few indeed, nevertheless the body possesses means of defense against many such poisons, which decrease to greater or less degree their harmful effects. True immunity, associated with the production of neutralizing substances in the blood, has as yet been obtained only against substances of proteid nature or substances very closely resembling the proteids. Studies on bacterial immunity and allied topics have as yet shown nothing to explain the acquirement of tolerance to morphine, alcohol, arsenic, and other similar poisons. A few observers have claimed that the serum of animals immunized to morphine will neutralize to some degree the toxic effects of morphine, but these results have not been generally substantiated.¹ Others have claimed that increased oxidative powers are developed under the stimulation of the poison which permits of its more rapid destruction, especially in the liver, but the experimental support of this hypothesis is slight. Still another idea is that, at least in the case of morphine, decomposition products are produced and accumulate in the body that neutralize physiologically to some extent the morphine itself; this hypothesis can scarcely be applied to arsenic and alcohol tolerance.²

It is possible, also, that the cell constituents with which the poisons ordinarily combine are produced in increased amounts under the stimulus of the poison, just as they are in the case of immunization with toxins, with the difference that the combining substances are not thrown off into the blood. For example, it has been claimed that arsenic is ordinarily combined and held in the liver by a nucleoproteid, and the suggestion has been made that in arsenic habitues this nucleoproteid is increased in amount. Again, saponin seems to act upon the cholesterin of the red corpuscles, and Kobert observed some resistance to the action of saponin exhibited by the serum of

¹ See Morgenroth, *Berl. klin. Woch.*, 1903 (40), 471.

² Concerning immunity against morphine see Faust, *Arch. exp. Path. u. Pharm.*, 1900 (44), 217; and Cloetta, *ibid.*, 1903 (50), 453.

immunized animals, which he attributes to an increased amount of cholesterin, perhaps liberated by the corpuscles decomposed by the injected poison, or perhaps produced in excess by the tissues. Wohlgemuth¹ has also suggested that in the case of poisoning with large amounts of substances which combine with glycuronic acid (*e. g.*, lysol), excessive quantities of this substance are formed by the cells and excreted into the blood, where they neutralize the poisons in much the same manner as the antitoxins neutralize toxins.

But besides these scanty examples of tolerance to poisons the body possesses a number of methods for opposing many other poisons with more or less success; and, poisons invariably acting chemically, the defenses are in turn largely chemical. We have elsewhere referred to the destructive action of the enzymes of the digestive tract upon bacterial and similar poisons; this means of defense cannot apply to ordinary chemical substances, because of their non-proteid nature. But the acidity of the gastric juice, the alkalinity of the bile and pancreatic juice, and the precipitating effect of the hydrogen sulphide formed in intestinal putrefaction are all factors that help to neutralize or prevent the absorption of certain poisons, their total efficiency, however, being on the whole very slight. After absorption of a poison a large series of chemical reactions and physiological processes is brought into play, and there are few poisons indeed whose harmful influence is not more or less decreased by these means. Kobert² classifies these protective processes as follows:

1. **Rapid elimination**, either before absorption by means of diarrhea and vomiting, or by the same means after absorption in case the poisons are excreted into the digestive tract (*e. g.*, morphine, venoms, antimony, and many other metals). Many poisons are very rapidly eliminated by other routes (*e. g.*, anesthetics, curare), in some instances causing harm, particularly to the eliminating organ (*e. g.*, kidneys in phenol poisoning, intestines in ricin poisoning). The routes and conditions of elimination of poison have been recently fully discussed by Lewin.³

2. **Deposition and Fixation in Single Organs or Tissues.**—In this respect the liver is especially important, probably because of its location and function as a filter for all the blood coming fresh from the alimentary tract. The manner and means

¹ Biochem. Zeitschr., 1906 (1), 134.

² "Lehrbuch der Intoxikationen," Stuttgart, 1902.

³ Deut. med. Woch., 1906 (32), 169; see also Mendel *et al.*, Amer. Jour. Physiol., 1904 (11), 5; 1906 (16), 147 and 152.

by which this fixation is brought about are unknown. According to Slowtsoff¹ arsenic is fixed by the nucleus in a very firm combination; ² mercury by globulins in a less stable combination; copper by the nucleins, but less firmly than the arsenic. Other poisons, chiefly alkaloids, are probably combined with bile acids. Possibly some poisons combine with *glycogen*. These compounds are but slowly broken up, and thus the poison reaches the more susceptible and more important tissues in a relatively diluted condition. The bones seem to hold in harmless form poisonous fluorides, and to less extent arsenic, barium, and tungsten, which persist in the bones for a great length of time. Leucocytes are possibly important binders of poisons, perhaps through combination with their nucleins,³ but storage in these labile cells is necessarily of relatively brief duration. Many poisons combine with the inorganic constituents of the tissues; *e. g.*, barium and various aromatic substances with SO_4 ; silver with Cl , etc.

3. **Combination** with substances formed or contained in the tissues; the resulting substance being less toxic than the poison alone. This method will be considered at greater length in connection with the related, often associated, method of defense; namely:

4. **Chemical alteration**, with or without subsequent combination with other substances, by such means as oxidation, reduction, hydrolysis, and neutralization.

5. **Impaired absorption** should also be considered as a means of defense against poisons. This may depend upon the injury to the gastro-intestinal tract produced either by the poison itself or by some independent pathological condition. Cloetta considers impaired absorption important in acquired immunity to arsenic (see below) and it may also modify the effects of other poisons.

The chemical reactions employed in defense against simple chemical poisons have been particularly considered by E. Fromm,⁴ whose outline is here partially followed, and to which the reader is referred for bibliography.

INORGANIC POISONS

Metallic poisons, such as lead, silver, mercury, and arsenic, are made insoluble, particularly by forming compounds with proteids in the alimentary tract, intestinal walls, blood, or internal

¹ Hofmeister's Beitr., 1901 (1), 281; 1902 (2), 307.

² Denied by Heffter (Arch. internat. de Pharmacodyn., 1905 (15), 399), who considers it more a physico-chemical process.

³ Stessano, Compt. Rend. Acad. Sci., 1900 (131), 72.

⁴ "Die chemischen Schutzmittel des Tierkörpers bei Vergiftungen," Strassburg, Karl Trübner, 1903. See also résumé by Ellinger, Deut. med. Woch., 1900 (26), 580.

organs; also by forming sulphides with the H_2S of the intestinal contents. According to Cloetta¹ immunization against arsenic depends entirely upon a reduction of absorption in the intestine, for the longer arsenic is taken, the less appears in the urine and the more appears in the feces. At the same time the resistance to arsenic injected subcutaneously is not increased at all, and no increase in resistance can be obtained by repeated subcutaneous injections of sublethal doses.

Free acids and alkalies are partly neutralized by the alkaline and acid contents of the gastro-intestinal tract, partly by forming compounds with the proteids, and partly by the alkalies and carbonic acid of the blood stream. (See "Acid Intoxication," Chap. xviii.) Phosphorus and sulphides are oxidized after absorption into phosphoric and sulphuric acid, which are in turn neutralized by the alkalinity of the blood and tissues. Lillie² has called attention to the close, palisade arrangement of the nuclei of the epithelium lining the alimentary tract, which makes it necessary for all substances absorbed to pass through the zone of their active oxidative influence, a fact undoubtedly of great importance in the defense of the body.

Reduction of iodic acid, chloric acid, hypochlorous acid, and their salts occurs in the body, resulting in their conversion into the much less toxic iodides and chlorides. Tellurium compounds are also reduced and rendered insoluble. This reaction occurs to some extent in the intestines; how much in other organs is unknown.

Methylation, the addition of CH_3 groups, is observed in poisoning by tellurium, which is eliminated in the breath as methyl telluride, and also in the sweat and feces.³ Selenium, pyridine, and some other substances also combine with methane. The source of the methane is possibly in the xanthin molecule.

Summary.—There are, therefore, three chief reactions used against inorganic poisons in the body, *oxidation*, *reduction*, and *splitting off of water*; neutralization of acids or alkalies and the formation of albuminates and sulphides being included under the last heading, since in these reactions the splitting off of water is an essential step.

ORGANIC POISONS

In the case of organic poisons an equally small number of primary reactions is employed in their detoxication, but in

¹ Arch. exp. Path. u. Pharm., 1906 (54), 196.

² Amer. Jour. Physiol., 1902 (7), 412.

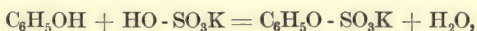
³ See Mead and Gies, Amer. Jour. Physiol., 1901 (5), 105.

more complicated manners and combinations corresponding with the complexity of organic compounds.

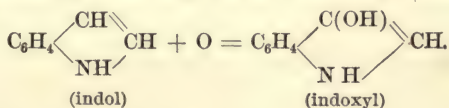
Oxidation, which has already been mentioned as a means of destruction of bacterial toxins, is naturally one of the most effective agents in the destruction of simpler organic substances, since the ordinary decomposition of all organic food-stuffs is through oxidation. There are numbers of specific examples of the conversion of a poisonous into a less poisonous or non-poisonous substance by oxidation. All acids of the fatty acid series are oxidized vigorously in the body, eventually into CO_2 and H_2O ; and occasionally pathologically produced oxalic, acetic, and lactic acids are destroyed in this way. Uric acid is oxidized vigorously by many organs, as are other members of the purin series, such as caffein and theobromin. Presumably oxidation of organic poisons as well as of food-stuffs is brought about by the oxidizing enzymes of the cells, as shown by Ehrlich's *indophenol reaction*, which consists of the oxidation of paraphenylene diamine and α -naphthol, with a resulting synthesis. This reaction has been shown by Lillie¹ to occur principally in and about the cell nuclei.

Combination, with or without Preliminary Oxidation.—Oxidation is also an essential preliminary step to many of the protecting combinations, in which a cell constituent is united to an organic poison. The most important of these combining substances are :

1. **Sulphuric Acid.**—One of the earliest and most important observations of the protective action of sulphuric acid was made by Baumann and Herter,² who showed that phenol is eliminated as a potassium salt of the sulphuric acid derivative, as follows :

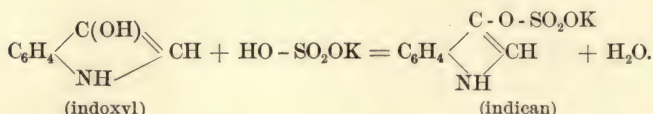


a reaction that has been of much practical use in treating phenol poisoning. As phenol and cresols are produced constantly in intestinal decomposition, this reaction is undoubtedly of great service, since the salt formed is relatively harmless. *Indol* and *skatol* are similarly detoxicated by being converted into corresponding salts, but only after a preliminary oxidation into *indoxyl* and *skatoxyl*, according to the following reaction :



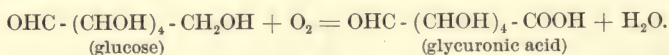
¹ *Loc. cit.*

² Zeit. physiol. Chem., 1877 (1), 247.



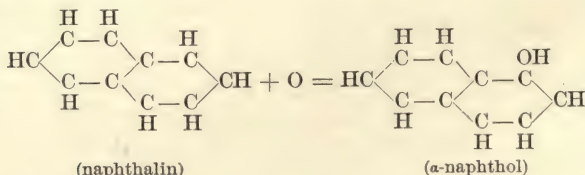
A host of other aromatic organic substances are similarly combined with sulphuric acid,¹ with or without preliminary oxidation, including all substances resembling phenol or which through oxidation are changed into phenols, such as cresol, thymol, anilin, naphthalin, pyrogallol, and tannin. By this means a poisonous substance is converted into a relatively harmless one, which is readily soluble and rapidly eliminated.

2. **Glycuronic acid** occupies the same position as sulphuric acid, combining particularly with naphthol, thymol, camphor, chloral hydrate, and butyl chloral. Sometimes a substance may appear in the urine combined in part with sulphuric, in part with glycuronic acid, showing the similarity of their function. Apparently when there is not sufficient sulphuric acid in the body to combine with all the poison, the excess unites with glycuronic acid,² although combination between glycuronic acid and the aromatic substance begins to occur before all the sulphuric acid is exhausted.³ Glycuronic acid represents merely a first step in the oxidation of glucose, as follows :



This oxidation occurs after the aldehyde group of the glucose has been combined by some other substance ; hence the aldehyde group escapes oxidation, although ordinarily more easily oxidized than the alcohol group.

Just as with the addition of sulphuric acid, oxidation may be a preliminary step to the addition of glycuronic acid ; *e. g.*, naphthalin is oxidized into α -naphthol, before uniting to glycuronic acid, as follows :



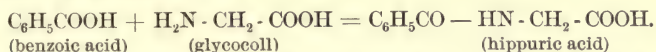
¹ See Hammarsten's Text-book (fourth American ed.), p. 542.

² See Austin and Barron, Boston Med. and Surg. Jour., 1905 (152), 269. Wohlgenuth has observed a case in which all the sulphuric acid of the urine was in organic combination (Berl. klin. Woch., 1906 (43), 508).

³ See Salkowski, Zeit. physiol. Chem., 1904 (42), 230.

The same is the case with many camphors and terpenes. Reduction may be the preliminary step, as with chloral hydrate, which is first reduced to trichlor-ethyl-alcohol. In still other cases splitting off of water is the chief preliminary step.

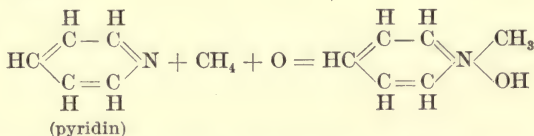
3. **Glycocoll** is one of the earliest known combining substances, the observation of the combination of glycocoll with benzoic acid to form *hippuric acid* being the first proof of synthesis in the animal body discovered by Wöhler (1824). The reaction is as follows :



A special enzyme has been found in kidney substance which can bring about this reaction outside the body. Normally this enzyme occurs chiefly in the kidney but may also occur in other organs. Many other aromatic compounds also combine with glycocoll before elimination, *e. g.*, salicylic acid. Some are first altered to a suitable form by oxidation; *e. g.*, toluol is oxidized to benzoic acid, xylol to toluic acid, nitro-benzaldehyde to nitro-benzoic acid. Many of the substances that can be made to combine with glycocoll in the body are of such a foreign nature that they never could need neutralization under any other than experimental conditions, but here, as with the sulphuric and glycuronic acid reactions, combination occurs whenever a suitable substance is present in the blood, glycocoll always being abundant as a cleavage product of the proteids.

4. **Urea** may also be a means of defense, forming salts with organic acids which are rapidly eliminated; *e. g.*, amido-benzoic acid and nitro-hippuric acid.

5. **Methane**.—Methylation, which occurs also with tellurium, is observed on administration of pyridin, as shown by the following equation :



This reaction is of special importance, because many alkaloids contain a pyridin group; and the resulting methyl compound may be less toxic than the original alkaloid—*e. g.*, methyl morphine.

6. **Sulphur** split off from proteids may combine with CNH and CNK, converting them into the much less toxic sulphonyanides.¹

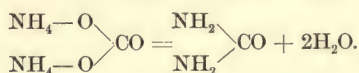
¹ See Meurice, Arch. int. Pharmacodyn., 1900 (7), 11.

7. Bile Acids.—All the above-mentioned reactions are protective largely because the substances formed are soluble and rapidly eliminated, as well as being less toxic than the original poison. Compounds of many poisons are formed with bile acids which are insoluble, and therefore only slowly dissolve or decompose, thus protecting the body from overwhelming doses of the poison. Such compounds are formed, not only with inorganic poisons, but also with alkaloids, especially strychnin, brucin, and quinin. They are then deposited in the liver, to be slowly dissolved and eliminated.

Occasionally *acetic acid* and *cystein* have been observed to act as combining substances.

Neutralization of organic acids entering the body or formed in metabolism is accomplished by the sodium carbonate of the blood when in small amounts; if excessive in quantity (*e. g.*, diabetic coma), a portion is combined with ammonia and appears as an ammonium salt in the urine. Magnesium and calcium salts may also help in the neutralization, probably at the expense of the bone tissue.¹ (See "Acid Intoxication," Chap. xviii.)

Dehydration, which plays a prominent part in a number of the above-mentioned syntheses, is particularly important in the change of ammonium carbonate into urea:



As ammonium salts of all sorts are very toxic, especially hemolytic, while urea is not, this process is probably one of the most important detoxicating reactions of the body because of the great amount of ammonium compounds that is constantly being formed in nitrogenous metabolism.

Summary.—As Fromm points out, the variety of reactions and the variety of defensive substances are both remarkably small in number. The reactions are: oxidation and reduction, hydration and dehydration, and perhaps simple addition (methylation). The chief known protective substances are the alkalies of the blood, proteids, hydrogen sulphide, sulphuric acid, glyco-coll, urea, cystein, bile acids, glycuronic acid, and acetic acid. *All these substances are normally present in the body, and none of them is specific against any one poison*, but each combines with several poisons. This last fact is interesting in comparison

¹ In this connection it may be mentioned that the bactericidal power of the blood is increased if the blood is more alkaline, decreased if it is less alkaline, than usual.

with the highly specific nature of the immune substances against bacteria and their products.

As far as we know, no particular increase in the neutralizing substances results from the administration of inorganic or organic poisons. The body does not appear to produce any excessive amounts of sulphuric acid in carbolic-acid poisoning or of glycocoll when benzoic acid is administered. Both substances are present in the body normally, and as much as is available combines with the poison; if there is not enough, the remaining poison combines with something else, or goes uncombined. In other words, the neutralizing substances described above do not appear to be the result of any special adaptation to meet a pathological condition. They are present in the body as a result of normal metabolism; they have an affinity for various chemical substances, some of which happen to be poisons; if these poisons happen to enter the body, they may be combined and neutralized to some extent, but, as a rule, very incompletely. There appears to be no elaborate process of defense against the chemically simple poisons, such as seems to be called into action by bacterial infection, and hence a degree of resistance or immunity similar to that present after an attack of scarlet fever or small-pox does not exist for strychnin or phosphorus.

CHAPTER VIII

PHYTOTOXINS AND ZOOTOXINS

THE production of substances possessing the essential features of true toxins is by no means limited to the bacterial cell. In the plant kingdom such substances are formed, and called *phytotoxins*. Of these, the best known are ricin, abrin, crotin, and robin. Among the toxins of animal origin, *zootoxins*, are the venoms of poisonous snakes, lizards, spiders and scorpions, and the serum of eels and snakes. These may be briefly considered as follows :

PHYTOTOXINS

The chief phytotoxins¹ are as follows :

Ricin, from the castor-oil bean (*Ricinus communis*).

Abrin, from the seeds of *Abrus precatorius*.

Crotin, from the seeds of *Croton tiglium*.

Robin, from the leaves and bark of the locust, *Robinia pseudoacacia*.

In their general properties all these substances are very similar and may be considered together. They resemble proteids in many respects, for they can be salted out of solutions in definite fractions of the precipitate, are precipitated by alcohol, and are slowly destroyed by proteolytic enzymes. For some time they were referred to in the literature as toxalbumins, until Jacoby stated that, by combining the salting-out method with trypsin digestion, he was able to secure preparations of ricin and abrin that did not give the proteid reactions. This seemed to place them in the same category with bacterial toxins and enzymes, *i. e.*, large molecular colloids, closely resembling the proteids with which they are associated, but still not giving the usual proteid reactions. Because of their great similarity to bacterial toxins this seemed a very probable description, and it has been generally accepted. More recent work by Osborne, Mendel, and Harris² however, does not support Jacoby's contention. They found the toxic properties of ricin associated inseparably

¹ Résumé of literature by Jacoby, Biochem. Centralblatt, 1903 (1), 289.

² Amer. Jour. of Physiol., 1905 (14), 259.

with the coagulable albumin of the castor beans, and were able to isolate it in such purity that one one-thousandth of a milligram (0.000001 gram) was fatal *per kilo* of rabbit, and solutions of 0.001 per cent. would agglutinate red corpuscles. The toxicity was also impaired or destroyed by tryptic digestion. They consider that probably, because of its extremely great toxicity, Jacoby was able to get active preparations that contained too little active substance to give the proteid reactions. As they remark: "If one-thousandth of a milligram of a compound giving on analysis every indication of being a relatively pure protein, is physiologically active in the degree characterized by our experiments, the toxicity of any impurity must be infinitely greater than that of any known toxins." In this connection may be mentioned the ultra-microscopic studies of tetanus antitoxin quoted by v. Behring¹ which showed that by dialysis a solution containing active antitoxin could be obtained giving none of the reactions for proteids, yet by ultra-microscopic methods it could be demonstrated that proteids were present. Against the claim that the toxic principle is simply carried down with the proteid is the fact that it does not come down in the first fraction that is precipitated, the globulin, which usually carries down all impurities. All the ricin comes down between the limits of one-fifth and one-third saturation with ammonium sulphate, exactly as does the albumin.

Immunity.—The phytotoxins have been very servicable in the study of immunity, since they obey the same laws as bacterial toxins and can be handled in more definite quantities. By their use Ehrlich first determined that toxin and antitoxin act quantitatively. They seem to possess haptophore and toxophore groups, and immunity is readily obtained against them, not only by subcutaneous injection, but by dropping into the conjunctival sac, and also by feeding, showing their direct absorbability and their resistance to digestion. The antitoxin is present in the milk of the immunized mother and immunizes the suckling; but little is carried through the placenta into the fetal blood. The immunity is specific, ricin antitoxin, for example, not protecting against abrin (although it is said to protect against robin). Roemer found that in animals immunized by conjunctival application the eye so used became immune to the local action of the poison before the other eye did, indicating a local development of immune substance. In general immunization the immune substance appears first in the spleen and bone-marrow. Normal serum gives a precipitate with ricin, but immune serum

¹ Beitr. exp. Therapie, 1905, H. 10, p. 22.

gives a much heavier one. Antiricin, like other antitoxins, is inseparable from the proteids of the serum.

Physiological Action.—Their poisonous action is manifold, most prominent being agglutination of the erythrocytes, local cellular destruction, and, to a less extent, hemolysis. Jacoby believes that in ricin there are several toxic substances differing in physiological properties, similar to Ehrlich's findings in diphtheria toxin (toxones, etc.). By long action of pepsin-HCl upon ricin, he secured a preparation with all the other properties of ricin except that it was inactive against erythrocytes; the same result could not be obtained with abrin. Heating to 65° or 70° does not destroy the toxicity of phytotoxins, but boiling does. There is a latent period of several hours after injection of the poison, the onset of symptoms being sudden; death rarely occurs in less than fifteen to eighteen hours (Osborne *et al*).

Flexner¹ has studied particularly the histological changes produced by ricin and abrin poisoning in animals. Both act alike, affecting the tissues much as bacterial toxins do (diphtheria). Fever, albuminuria, and convulsions are followed by exhaustion and lowered temperature. Punctiform hemorrhages are found beneath the serous surfaces, with fluid in the peritoneal cavity. At least in the case of ricin the hemorrhages are not due to blood changes, but to a special toxin destroying the endothelial cells.² There occur a general lymphatic enlargement and marked changes in the intestinal mucosa, with swelling of the Peyer's patches. The spleen is swollen and dark in color, as also is the liver, which shows much focal necrosis. Subcutaneous injection causes local edematous inflammation without suppuration. Histologically, in the most affected organs are found much cellular necrosis and disintegration, especially of lymphoid and epithelial cells. Changes in the capillary endothelium, fibrinous thrombi, and abundant hemorrhagic extravasations are wide-spread. Probably agglutinative thrombosis by red corpuscles plays an important part in these intoxications (Ehrlich). The great amount of intestinal injury probably depends upon the fact that these poisons are largely eliminated through the intestinal mucosa.

Mushroom Poisons.—The poisons of the three chief poisonous mushrooms, *Amanita muscaria*, *Helvella esculentia*, and *Amanita phalloides*, differ from one another quite essentially. The poisonous principles of the first and second, muscarin and helvellic acid, are non-proteid substances, of known chemical composition, which are discussed else-

¹ Jour. Exper. Med., 1897 (2), 197.

² Amer. Jour. Med. Sci., 1903 (126), 206.

where ; but the *Amanita phalloides*, the most important of the three, owes its toxic properties to substances which, according to the investigations of Ford,¹ are true phytotoxins. At least two poisonous constituents are present in *A. phalloides*. One, the *phallin* of Kobert, is powerfully hemolytic, is destroyed by heating thirty minutes at 65°, and acts directly upon red corpuscles without the presence of serum, thus resembling the bacterial hemolysins. Phallin is also destroyed by the action of pepsin and pancreatin. This agent produces the subcutaneous edema, hemoglobinuria, and pigmentation of the spleen observed in animals into which it has been injected.

The hemorrhages, necrosis, and fatty degeneration observed in poisoned animals are due to another distinct poison, which Ford calls *amanitotoxin*. This poison is thermostable, not being destroyed by the temperature that destroys the activity of phallin (65°), but it does not resist heating to 90° or over. It also differs from phallin in being resistant to pepsin and pancreatin. Animals can be immunized against amanita extracts, and their serum will neutralize the poisons. Both poisons resemble the bacterial toxins in possessing toxophore and haptophore groups, which are quite distinct in each poison, since immunization with the thermostable body gives a serum that has no neutralizing effect upon phallin.

THE TOXIN CAUSING HAY-FEVER

In 1902 Dunbar² demonstrated conclusively that typical hay-fever, in its several various forms, is due to pollen of various sources, in all, twenty-five varieties of grass and seven varieties of plants of other sorts being found whose pollen, when placed upon the nasal or conjunctival mucous membranes of hay-fever patients, cause a typical attack of the disease. In Germany the disease seems to come chiefly from pollen of the grasses and grains (rye pollen being most active), whereas in America the most important pollen seems to come from members of the *Ambrosia* (rag-weed) and *Solidago* (goldenrod). Dunbar also found that the toxic constituent could be dissolved from the pollen in salt solution, and seemed to be a proteid. The proteid constituents of the pollen of rye have been studied further by Kammann,³ who found three proteids, one of which, an albumin, was found to contain all the toxic matter. This constitutes about 5.5 per cent. of the entire weight of the pollen, is weakened but little by heating to 80°, and is not destroyed by boiling ; it is but partly destroyed by pepsin and trypsin, and resists acids but not alkalis. So toxic is the material that a solution containing $\frac{1}{1200}$ milligram of pollen proteid, which amount is contained in two or three pollen grains, produces a reaction in susceptible individuals, but large amounts have no effect on normal persons. Dunbar has manufactured an anti-toxic serum⁴ by immunizing horses against the pollen, which seems to

¹ Jour. Infectious Diseases, 1906 (3), 191 ; Jour. Exp. Med., 1906 (8), 437.

² Full review of subject and literature given by Glegg, Jour. of Hygiene, 1904 (4), 369 ; concerning etiology see Liefmann, Zeit. f. Hygiene, 1904 (47), 153 ; also Wolff-Eisner, Deut. med. Woch., 1906 (32), 138, and "Das Heufieber," München, J. F. Lehmann, 1906.

³ Hofmeister's Beitr., 1904 (5), 346.

⁴ Wolff-Eisner does not consider the toxic substance a true toxin, but Kammann (Berl. klin. Woch., 1906, p. 873) upholds Dunbar's view that it is a true toxin, and that the anti-serum contains a true antitoxin.

produce decided therapeutic effects, although by no means all observers are agreed as to its efficacy.¹

(The effects of the phytotoxins on the blood are discussed under "Hemolysis" in the following chapter. Vegetable hemolytic poisons that do not resemble the toxins, *e. g.*, glucosides, etc., will also be found discussed under the same heading.)

ZOOTOXINS²

SNAKE VENOMS

This important class of poisons, first thoroughly investigated by Weir Mitchell (1860), and Mitchell and Reichert (1883), has recently aroused great interest through its relations to bacterial toxins and the problems of immunity. The poisons of different species of snakes seem to have much in common with one another, whether derived from the *Elapérine* snakes (cobras and numerous other Indian and Australian snakes), or *Viperidae* (including most poisonous American snakes), or *Hydrophinae* (the poisonous sea-snakes), although very characteristic differences exist between each.

The essential anatomical differences between the different classes of snakes are as follows: *Colubridae*, which include all the non-poisonous snakes, have no mechanism for injecting poisons into their victims. *Colubridae venenose* are venomous snakes resembling in many particulars the harmless Colubrines, but having short poison fangs, firmly fastened to the maxilla in an erect position; in this class are included the cobra and the venomous snakes of Australia. *Viperidae*, or vipers, are characterized by a highly specialized apparatus for injecting the poison; their poison fangs are very long, and the maxillary bone, to which they are fastened, is so articulated that it rotates about a quarter of a circle when the snake strikes, bringing the fangs into an erect position. The fangs are canalized and pointed at the end like a hypodermic needle, and the poison is forced through them under considerable pressure by a large muscle that contracts over the salivary gland. Accessory fangs in various stages of development are also present to replace any fang lost in action. All the poisonous snakes of North America, with one insignificant exception, belong to the vipers, and to a special class known as the "pit vipers," because of the presence of a deep pit of unknown function above the maxilla. The exception mentioned is the "coral snake" found on the coast of Florida, around the Gulf of Mexico and in the southeastern states; it is a member of the colubrine poisonous snakes, of small size, and seldom causes serious poisoning. The poisonous vipers are the rattlesnakes (*Crotalus*), of which there are some ten to twelve or more species, and *Sistrurus*, of which there are two species;

¹ See Ingals, Jour. Amer. Med. Assoc., 1906 (47), 376.

² Full review and literature given by Faust, "Die tierischen Gifte," Braunschweig, 1906. Hemolytic Properties of Animal Poisons, discussed by Sachs, Biochemisches Centralblatt, 1906 (5), 257.

the copperheaded adder (*Ancistrodon contortrix*) and the water moccasin (*Ancistrodon piscivorus*).

(The classification used above is the one followed in most publications on poisonous snakes; a more modern classification divides the snakes (*Ophidia*) into several series, one of these including all poisonous snakes under the title of *Proteroglypha*, and dividing this series into the three families: (1) *Elapinae*, including cobras, coral snakes, etc.; (2) *Hydrophinae*, the poisonous sea-snakes; (3) *Viperidae*, including all snakes with erectile fangs.¹)

The source of the venom is probably in part the blood, since snake blood has been found to contain poisons very similar to some of those in the venom; therefore these are presumably simply filtered out by the venom glands, and not manufactured by them. Other poisonous constituents of venom are not found in snake serum, and therefore are probably manufactured by the venom gland. Apparently many of the harmless snakes produce a poisonous saliva, since extracts of their glands are said by Blanchard² to possess the properties of the venoms, and if so these snakes are harmless chiefly because they lack an apparatus for injecting the poison. As a rule, however, the venom glands are much more highly developed in the poisonous snakes, and are connected with a specialized injection apparatus; in structure they are compound racemose glands.

Properties of Venom.—As ejected, the venom is weakly acid or neutral in reaction, and free from bacteria, contrary to earlier ideas (Langmann). Its specific gravity is 1030 to 1077, and it contains a large amount of solids, generally 20 to 30 per cent. by weight. These are precipitated by alcohol, ether, tannin, and iodine, but do not adhere to precipitates of phosphates as do enzymes and toxins (Calmette). They do not diffuse through dialyzing membranes. When dried, the venom can be kept almost indefinitely without losing its strength, specimens over twenty years old having been found unimpaired. Glycerin and alcohol also seem not to injure it, but oxidizing agents of all kinds are very destructive. Light impairs the power of venoms, as also does radium (Phisalix³). Eosin and erythrosin also reduce the power of venom through their photodynamic

¹ For a full discussion of the characteristics of the poisonous snakes of North America, see the monograph with that title by Stejneger, Report of U. S. National Museum, 1893, Washington. A good summary is also given by Langmann, Reference Handbook of Medical Sciences, vol. 8, p. 708. Concerning poisonous sea-snakes, *Hydrophidia*, see Boulanger, Natural Science, 1892 (1), 44. The poisonous snakes of India are described by Fayrer, in "The Thanatophidia of India," London, 1874.

² Compt. Rend. Soc. Biol., 1894 (46), 35.

³ Compt. Rend. Soc. Biol., 1904 (56), 327.

action, affecting the neurotoxic properties less than the hematoxic components (Noguchi¹).

Much work has been done upon the nature of the constituents of venom. As early as 1843 Prince Lucien Bonaparte found that there were proteids in the venom, which was corroborated by Mitchell in 1861. In 1883 Mitchell and Reichert described two poisonous proteid constituents of venom, one of which was coagulable by heat and seemed to be a globulin; the other resembled the proteoses (they called it "peptone," according to the nomenclature of that time). To the globulin they ascribed the local, irritating properties of venom; to the albumose, the systemic intoxication. Corresponding to their action, venoms of different serpents were found to vary greatly in the proportions of these proteids. Cobra venom, which acts chiefly systemically, contains 98 per cent. of albumose and but 2 per cent. of globulin; rattlesnake venom, with its marked local effects, contains 25 per cent. of the irritating globulin; moccasin venom contains 8 per cent. of globulin. Several other observers soon corroborated the main facts of Mitchell and Reichert's report; but, as has been seen in connection with the consideration of the composition of enzymes, toxins, etc., the fact that a substance is carried down with a proteid is no proof that it is itself a proteid.² What has been established is merely that the irritating component of venom can be destroyed by heat, and is removed with the globulin in fractional separation; while there remains a substance not destroyed by boiling, which comes down at least in part with the albumoses of the venom, and causes chiefly systemic manifestations.

Enzymes in Venoms.—As venom causes rapid liquefaction of tissues into which it is injected, Flexner and Noguchi³ tested crotalus and cobra venom for proteases, and found that they digested muscle rapidly, and also gelatin and unboiled fibrin; whereas boiled fibrin and boiled egg-albumen were undigested. Wehrmann⁴ found that venom (cobra?) digests fibrin and inverts saccharose, but does not digest starch. Martin⁵ found fibrin ferments in various venoms.

Toxicity.—Calmette has determined the toxicity of several venoms, and gives the following figures:

¹ Jour. Exper. Med., 1906 (8), 252.

² Faust ("Tierische Gifte," p. 60) has described a non-proteid, nitrogen-free poison in cobra venom which he calls "ophiotoxin." It has a curare-like action and also paralyzes the central nervous system. Its general properties resemble those of picrotoxin and sapotoxin.

³ Univ. of Penn. Med. Bull., 1902 (15), 360.

⁴ Ann. d. l'Inst. Pasteur, 1898 (12), 510.

⁵ Jour. of Physiol., 1905 (32), 207.

1 gm. <i>cobra</i> or <i>aspis</i> kills	4000	kgm. of rabbit.
1 gm. <i>hoplocephalus</i> kills	3450	" " "
1 gm. <i>fer de lance</i> or <i>pseudechis</i> kills	800	" " "
1 gm. <i>Crotalus horridus</i> kills	600	" " "
1 gm. <i>Pelias berus</i> kills	250	" " "

The danger of the bite depends not only upon the difference in the strength of the venom of different varieties of serpents, but also upon the size of the snake, the time of year and condition of hunger or plenty, and particularly whether the entire discharge is injected successfully or not. Probably in the majority of strikes, by no means all the fluid ejected by both fangs is injected beneath the skin of the victim. A large diamond rattler may eject as much as a teaspoonful of venom at one discharge and such a dose would usually be fatal. Repeated ejections decrease the strength of the venom rapidly, until it may have almost no toxicity. In general, venom is most active in warm weather and immediately after the snake has fed; in winter its toxicity is slight.

The mortality in America from snake-bites is very hard to ascertain, various authors giving figures at wide variance. Weir Mitchell gives one series with a mortality of 25 per cent., and another series in which it was 12 per cent.; Ellzey gives 15 per cent. These figures are probably high, since fatal cases are much more likely to find their way into the literature than those in which the results are trifling. Some authors go so far as to say that there are no authentic cases of death from copperheads or moccasins, but this is undoubtedly incorrect.¹ However, the reputed danger from these snakes is undoubtedly much exaggerated, many deaths from snake-bites of all kinds being due to the treatment rather than to the bite. The poisonous snakes of Australia, although numerous, are not very virulent, and the mortality is given as about 7 per cent. A full charge of venom from the cobra and many other Indian snakes is inevitably fatal (Fayrer). The crotaline snakes of the tropics are more venomous than those of the north, *Lachesis lanceolatus* of Central America and Mexico being nearly as dangerous as the cobra.

When venom is taken into the stomach in the intervals of digestion, enough may be absorbed to produce death, especially in the case of those venoms which contain a large proportion of the albumose, which is dialyzable; but during active digestion the venom undergoes alteration and is rendered harmless. It has been found experimentally in animals that cobra venom

¹ Concerning copperhead poisoning see Yarrow, Amer. Jour. Med. Sci., 1884 (87), 422.

placed in the stomach causes ordinarily no harm whatever, but if a loop of the intestine is isolated, a fistula established and allowed to heal, venom introduced through this opening always produces death. It is probably not the pepsin and hydrochloric acid that destroys the venom, but the trypsin. If the bile-duct is ligated, the venom is destroyed just the same. Much of the venom seems to be eliminated into the stomach, no matter how it is introduced into the system, and apparently it is also partly excreted by the kidneys. Rattlesnake venom seems not to be absorbed through mucous membranes.

Physiological Action.—As indicated in the preceding paragraph, the effects of the bites of different classes of snakes are quite different. Langmann describes the symptoms as follows :

Cobra Poisoning.—“Within an hour, on an average, the first constitutional symptoms appear: a pronounced vertigo, quickly followed by weakness of the legs, which is increased to paraplegia, ptosis, falling of the jaw with paralysis of the tongue and epiglottis; at the same time there exists an inability to speak and swallow, with fully preserved sensorium. The symptoms thus resemble those of an acute bulbar paralysis. The pulse is of moderate strength until a few minutes after the cessation of respiration; the latter becomes slower, labored, and more and more superficial until it dies out almost imperceptibly. Death occurs at the latest within fifteen hours; in 32 per cent. of all cases in three hours. There are very few local changes.”

Viper Poisoning.—“After the bite of a viper the local changes are most pronounced; there are violent pains in the bleeding wound, hemorrhagic discoloration of its surroundings, bloody exudations on all the mucous membranes, and hemoglobinuria. Usually somewhat later than in cobra poisoning constitutional symptoms develop; viz., great prostration with nausea and vomiting, blood pressure falls continuously, and respiration grows slow and stertorous. After a temporary increase in reflexes, paresis supervenes, with paraplegia of the lower extremities, extending in an upward direction and ending in a complete paralysis. It therefore resembles an acute ascending spinal paralysis. If the patient recovers from the paralysis, a septic fever may develop; not rarely there remain suppurating gangrenous wounds, which heal poorly.”

It will be noticed that there is lacking the usual period of incubation that follows injection of bacterial toxins, and if it happens that the venom has been injected directly into one of the veins, death may occur within a few minutes. When recovery occurs, the disappearance of symptoms is remarkably abrupt, within a few hours a desperately sick person becoming almost entirely free from all evidences of the intoxication.

Pathological Anatomy.—*Postmortem examination* shows changes varying with the nature of the poisonous snake that has caused death.

In the case of a cobra bite, according to Martin, the areolar tissue about the wound is infiltrated with pinkish fluid; the blood is often fluid; the veins of the pia are congested, and the ventricles often contain turbid fluid; the kidneys may show much congestion. When death occurs in a few minutes, enormous general intravascular clotting is found, which seems to be the cause of death. After death from a viper bite the site of the wound is the seat of intense edema and extravasation of blood; if in the muscles, these are much softened and disorganized. Hemorrhages are found in all organs and in the intestinal tract. If death occurs after several days it is generally because of sepsis, and shows the usual changes of this condition; in addition, as a rule, to marked gangrenous, ulcerative, and sloughing processes at the site of the bite.

Histologically there are found, in addition to innumerable hemorrhages in nearly all the organs, many vessels plugged with thrombi composed of more or less hemolyzed, agglutinated erythrocytes. The changes produced in the nervous tissue by the Australian tiger snake are described by Kilvington,¹ who found marked chromatolysis, the Nissl bodies breaking into dust-like particles, and eventually all stainable substance disappearing from the cytoplasm; the nucleus retains its central position, but often loses its outline and may disappear. The cells around the central canal of the cord are most affected. There are no inflammatory changes in the nervous system, and if death occurs very quickly there may be no microscopic alterations. Hunter² found similar changes in the Nissl bodies in both krait and cobra poisoning; in the medullated fibers he found the myelin sheath converted into ordinary fat. Nowak³ studied experimental animals, and found much fatty change in the livers, even if death occurred one-half hour after poisoning; also focal necrosis in the liver, acute parenchymatous alterations in the kidney, and pneumonic patches in the lungs.

Effects on the Blood.—There has been much discussion concerning the part played by the abundant and prominent intravascular clotting in causing death after snake-bite. Lamb⁴ states that when venoms are slowly absorbed the coagulability of the blood is decreased and it is found fluid after death, but when a fatal dose of venom (viper) is rapidly absorbed, clotting is increased and thrombosis is the chief cause of death. Martin has demonstrated very active fibrin ferments in snake venom (*loc. cit.*). It is highly probable, however, that many of the thrombi of venom poisoning are not produced by coagulation of fibrin, but by agglutination of the red corpuscles, which Flexner⁵ has shown can cause large clots in the heart and great vessels, as well as "hyalin" thrombi in the small vessels.

Nature of Venoms.—The varied effects produced by venoms have been found to be due to a number of poisonous elements which they contain, and which have been distinguished and separated from one another by Flexner and Noguchi.⁶

¹ Jour. of Physiol., 1902 (28), 426.

² Glasgow Med. Jour., 1903 (59), 98.

³ Ann. d. l' Inst. Pasteur, 1898 (12), 369.

⁴ Indian Medical Gazette, Dec., 1901.

⁵ Univ. of Penn. Med. Bull., 1902 (15), 324.

⁶ Jour. Exp. Med., 1903 (9), 257; Univ. of Penn. Med. Bull., 1902 (15), 345.

These are *hemotoxins* (*hemolysins* and *hemagglutinins*), *leucocytolysins*, *neurotoxins*, and *endotheliotoxins* (*hemorrhagin*). In another place (see "Hemolysis") the nature of the hemolytic agent is discussed, and the important observation of Flexner and Noguchi, that the venom hemolysin is in the nature of an amboceptor,¹ is described. Venom agglutinin is quite independent of the hemolysin, for it is destroyed by heating to 75°–80°, whereas the hemolysin is destroyed only partly at 100°. Agglutinin acts in the absence of serum complement, and therefore is not an amboceptor like the hemolysin; it is apparently more like the toxins in its nature. The agglutination of the corpuscles does not interfere with their subsequent hemolysis.

The leucocytotoxins are quite distinct from the hemolysins, for after saturating all the hemolytic amboceptors with red corpuscles, the venom still shows its effects on the leucocytes, which effects consist in cessation of motility and disintegration, affecting particularly the granular cells. The leucocytotoxin, however, resembles the hemolysin in that it appears to be an amboceptor. Leucocytes are also agglutinated by venom, possibly by the same agglutinin that acts on the red corpuscles.

By saturating venom with either red corpuscles or nerve-cells it is possible to prove that the toxic principle for each is distinct and separate. Other sorts of cells, however, are able to combine, or at least remove some parts of the toxic elements, but to a much less degree. The neurotoxin, like the hemolysin, is an amboceptor, and since venom contains no complement, the neurotoxin has first to be supplied with complement by the victim's blood before it can harm the cells. This is a remarkable example of a substance, the complement, which is normally intended for defense, acting as a toxic agent.

The pronounced hemorrhage-producing property of serums, particularly that of the rattlesnake, was also found to be due to a specific toxin acting on the endothelium of the capillaries and small veins, and not to the changes in the blood itself, as had formerly been thought. This endotheliotoxin, which Flexner and Noguchi call "hemorrhagin," is quite distinct from the other toxic substances, being destroyed at 75°, a temperature that leaves the neurotoxin and hemolysin uninjured.

Variations in Venoms.—In distribution among the various poisonous reptiles these toxins are also quite distinct from one another, which explains the difference in the effects of bites

¹ This use of the term hemolysin is usual, but not strictly correct, for the amboceptor by itself is not hemolytic. A more exact statement would be that the venom hemolysin is an amboceptor-complement complex (Ricketts).

by snakes of various kinds. Cobra venom contains chiefly neurotoxin, hence the symptoms of cobra bite are largely of nervous origin, with but little local tissue change. Rattlesnake venom owes its effects chiefly to hemorrhagin, hence the marked local necrosis and extravasations of the blood, and the generalized hemorrhages; the nervous effects following viper bite are probably, in part, due to hemorrhages in the nervous tissue. Cobra venom produces great hemolysis and little agglutination. Rattlesnake venom has relatively little agglutinative or hemolytic power. Water moccasin and copperhead venoms are more agglutinative than either, and intermediate in hemolytic strength; they cause much local tissue destruction.

The exact action of cobra venom on various centers and organs has been studied by Elliot.¹ It raises blood pressure when in dilution of 1 : 10,000,000, by contracting vessels and stimulating the heart; low lethal doses kill by paralyzing the respiratory center.

Krait (*Bungarus ceruleus*) venom acts similarly, but less powerfully, and cannot be neutralized by Calmette's antivenin.²

Sea-snake venoms are by far the most poisonous of all. For *Enhydrina valakadien* the lethal dose for rabbits is 0.00006 gram per kilo body weight. It acts by vagus stimulation and paralysis of respiratory centers and of motor nerve-endings.³

Russell's viper (*Daboia Russellii*) owes its effects chiefly to intravascular clotting, according to Lamb and Hanna,⁴ and contains no neurotoxin. It is not neutralized by Calmette's antivenin. The clots are due to agglutination and contain no fibrin (Flexner).⁵

The "Gila monster" (*Heloderma suspectum*) seldom causes serious poisoning in man, but may kill small animals, such as frogs. Its poison is only slightly hemolytic, but produces degenerative changes in the nervous system (Langmann).

Loss of Bactericidal Powers.—The frequency of marked and persistent sloughing and suppuration at the site of snake-bites, particularly from the vipers, and the common termination in sepsis, was attributed by Welch and Ewing⁶ to a loss of bactericidal power of the blood, which they found followed experimental venom poisoning. This has been nicely explained by Flexner and Noguchi as the result of saturation of serum complement by the numerous amboceptors of the venoms, so that no complement is left for the serum to use against the bacteria. In serum whose complements do not combine with the

¹ Lancet, 1904 (i), 715.

² Elliot, Sillar, and Carmichael, Lancet, 1904 (ii), 142.

³ Fraser and Elliot, Lancet, 1904 (ii), 141; also Rogers, Jour. of Physiol., 1903 (30), iv. The above are also given completely in the Philosophical Transactions of the Royal Society, 1904-5, vol. 187.

⁴ Jour. of Path. and Bact., 1902 (8), 1.

⁵ Thorough study by Van Denburgh and Wright, Amer. Jour. of Physiol., 1900 (4), 209.

⁶ Lancet, 1894 (1), 1236; Ewing, Med. Record, 1894 (45), 663.

venom amboceptors (*e. g.*, *Necturus*) the normal bactericidal powers are not in the least impaired by the addition of venom.

Snake Serum.—The *serum* of serpents is also toxic for other animals, even when the serpent is not a venomous one; *e. g.*, the harmless pine snake (*Pityophis cateniferis*). The toxicity of snake serum seems to depend chiefly upon its hemotoxic effects (hemagglutination and hemolysis), the toxic substances being in the nature of amboceptors and similar to, but not altogether identical with, the amboceptor of the venoms. *Crotalus* tissues also produce poisoning in proportion to the blood they contain, but are without toxic effects of their own (Flexner and Noguchi).

Antivenin.—Snake venom has the essential property of all true toxins of immunizing, with the appearance of an antitoxin in the blood. The first successful immunizations seem to have been made by Sewall,¹ but the practical production of antitoxin serum was first accomplished by Calmette² and by Fraser.³ This antivenin neutralizes the neurotoxins and hemolysins of venoms of *any* origin, and also of snake serums, and, therefore, is quite effective against cobra and similar venoms which produce chiefly neurotoxic and hemolytic changes. This indicates that these toxic substances are of identical nature in all snakes, no matter how dissimilar the snakes may be. Cobra antivenin does not, however, neutralize the hemorrhagin of rattlesnake venom, for the venoms used by Calmette do not contain this principle abundantly. A special antitoxin against rattlesnake venom and its hemorrhagic toxin has been successfully prepared by Noguchi.⁴ This *crotalus* antivenin also neutralizes hemolysins of all sorts of venoms, and also of snake serums.

Presumably antivenin neutralizes venoms in exactly the same way that antitoxin neutralizes toxins; *i. e.*, cell receptors are thrown off from the injured cells during immunization, which combine with venom amboceptors in the blood, and thus prevent their combining with the cells. Antivenin also prevents the inhibiting action of venom on bactericidal serum, indicating that it prevents the venom amboceptors from binding the serum complement. The reaction of venom and antivenin is certainly a chemical one, being likened by Kyes⁵ to that of strong acids upon strong bases.

¹ Jour. of Physiol., 1887 (8), 203.

² Ann. d. P. Inst. Pasteur, 1894 (6), 275; also subsequent articles in 1897 (11), 214; 1898 (12), 343.

³ British Med. Jour., 1895 (i), 1309.

⁴ Univ. of Penn. Med. Bull., 1904 (17), 154.

⁵ Berl. klin. Woch., 1904 (41), 494.

Lamb¹ found that antivenin for cobra acts as a *precipitin* for cobra venom, but considered it not specific for cobra venom, as it causes precipitation to varying degrees in other venoms. Hunter,² however, states that the precipitin is specific. It does not cause precipitation with cobra serum, but precipitins for cobra serum do precipitate cobra venom (Hunter). The precipitin formation is not essentially related to antitoxin formation. Flexner and Noguchi also observed that crotalus antivenin is strongly precipitating for crotalus serum, less so for crotalus venom, and but slightly for pine-snake serum; Calmette's antivenin is without precipitating action on either crotalus venom or serum.

As is well known, snakes are nearly or quite insusceptible to snake venom. Cunningham³ found that serum of cobras was devoid of antitoxic property, so the immunity of snakes must be ascribed to an absence of cell receptors in their tissues, with which their venom amboceptor can combine. The reputed immunity of the mongoose and hedgehog depends partly on a relatively low susceptibility, but probably more on the agility of the mongoose and the defensive spines of the hedgehog.

SCORPION POISON⁴

This poison is secreted by a pair of specialized glands in the posterior segment of the elongated abdomen, surrounded by a firm capsule with a sharp apex through which the poison is discharged. Its effect on man is usually confined to local pain, swelling, and occasionally phlegmonous inflammation with constitutional symptoms after bites from the largest species. In Africa a large scorpion (*Androctonus*) exists, that is reputed frequently to cause fatal poisoning, especially in children. The majority of serious results following scorpion bites, as well as bites of poisonous insects to be considered later, are, however, due to infection of the wound, which occurs readily because of local necrosis and hemorrhages, and also because of the unfavorable conditions existing in tropical climates. Apparently these bites favor local infection much as do those of vipers.

When general symptoms do occur, they are described as resembling strychnine poisoning, with trismus, stiffness of the neck and eventually of the respiratory muscles, which seems to

¹ Lancet, 1902 (ii), 431; 1904 (i), 916.

² Jour. of Physiol., 1905 (33), 239.

³ Nature, 1896 (55), 139.

⁴ A complete discussion of the literature on poisonous invertebrates, etc., is given by v. Fürth, "Vergleichende chemische Physiologie der niederen Tiere," Jena, 1903; and by Faust, "Die tierischen Gifte," Braunschweig, 1906.

be the chief cause of death (Cavorez). Thompson,¹ however, observed only seldom severe symptoms, consisting of general paralysis that passed off in a few hours. Most experimenters with scorpion poison describe it as chiefly a nerve-tissue poison, but it also seems to act as a hemolysin and agglutinin (Bellesme and Sanarelli). Calmette² gives the lethal dose for a guinea-pig as 0.5 milligram, while Phisalix and Varigny put it at 0.1 milligram and state that scorpion blood is also poisonous. Wilson³ found its toxicity equal to 0.1 gram per million, that is, one gram of poison will kill 10,000,000 grams of guinea-pig, hence it is much stronger than cobra venom. The average amount of toxin in an Egyptian scorpion (*Buthus quinque-striatus*) is sufficient to kill about 35 kilos, which agrees with the fact that fatal poisoning by this scorpion is rare in adults, but reaches 60 per cent. in children. The venom is harmless when taken into the stomach, and is said to be made inactive by ammonia, calcium hypochlorite, and peroxide of hydrogen. Calmette claims that antivenin for cobra in part neutralizes scorpion poison. A large number of naturalists and raconteurs have furnished interesting tales of suicide by scorpions, which are more than improbable in the light of our present knowledge concerning natural immunity.

SPIDER POISON

The poison apparatus of the spiders consists of two long pouches lying in the thorax and extending into the jaws, at the apex of which the poison is discharged. Some of the larger members of the family are very poisonous, *e. g.*, the Malmignatte (*Lathrodectes tredecimguttatus*), of the vicinity of the lower Volga in southern Russia, is said to have destroyed 70,000 cattle in one year, the bite being fatal in 12 per cent. of all cases, although rarely killing man. Other members of this species in Chili, Madagascar, and other countries are not much less venomous. Kobert has studied the poison of Malmignatte and found it distributed throughout the body of the spider, even in the eggs, and resembling in nature the snake venoms. It is destroyed by heating, and seems to be of proteid nature; the chief effect is upon the nervous system and heart.

A number of common spiders investigated by Kobert⁴ were

¹ Proc. Acad. Nat. Sci. of Philadelphia, 1886, p. 299.

² Ann. Inst. Pasteur, 1895 (9), 232.

³ Records of Egyptian Gov't., School of Med., 1904; abst. in Jour. of Physiol., 1904 (31), p. xlviii.

⁴ "Beiträge zur Kenntnisse der Giftspinnen," Stuttgart, 1901.

apparently not poisonous for mammals, except the "cross spider" (*Epeira diadema*), which has since been thoroughly studied by him and by Sachs.¹ In these spiders also the poison is found throughout the body. It resembles the snake venoms strikingly, according to Sachs, for it contains a powerful hemolysin which he calls "arachnolysin," acting very differently with different sorts of blood, and destroyed by heating at 70°-72° for forty minutes. Only such blood is hemolyzed as is able to bind the poison in the stroma of the red corpuscles. By immunizing a guinea-pig Sachs succeeded in securing an antitoxin of some strength. The discovery of this hemolysin explains Kobert's observation of hemoglobin, methemoglobin, etc., in the urine of persons bitten by spiders.

Von Fürth considers that the bite of the historically famous Italian tarantula is able to cause no more than local inflammation, and Kobert found that the entire extract of six Russian tarantulas (which are supposed to be more poisonous than the Italian) caused no symptoms when injected into a cat.

In all probability the other poisonous spiders possess toxic substances allied to those of the venoms, with hemolytic, agglutinative, and neurotoxic products, Sachs' studies indicating the general similarity of all the zootoxins.

CENTIPEDES

Undoubtedly the severity of centipede poisoning has been greatly exaggerated, the results being usually limited to local inflammation, frequently spreading some distance in an erysipelas-like manner. An authentic case of fatal poisoning of a child four years old by a centipede (*Scolopendra heros*) has been reported from Texas by G. Linceicum,² death resulting five to six hours after the bite was received. Besides the local pain and inflammation, vomiting was marked, occurring also in five other non-fatal cases.

Centipedes secrete their poison in relatively large glands, which discharge at the apices of a pair of specialized claws that take the place of the first pair of legs. The nature of this poison seems not to have been investigated. Numerous chemical substances are described as secreted by other glands of these animals, including prussic acid and a camphor-like matter (see v. Fürth).

¹ Hofmeister's Beitr., 1902 (2), 125.

² Amer. Jour. Med. Sci., 1866 (52), 575.

BEE POISON

Bee poison has been better studied than most insect poisons, beginning with the work of Paul Bert (1865). It is secreted by the glands into a small poison sac, and stored up until ejected. Cloez found that bee poison was precipitated by ammonia, tannin, and platinic chloride, and Langer proved it to be a non-volatile organic base. As excreted, it is acid, contains 30 per cent. of solids, and one honey-bee secretes 0.0003–0.0004 gm. It contains formic acid and much proteid, but it has been stated that the poison is proteid-free, and is not destroyed by heat (100°), weak acids, or alkalis. On the other hand, it is said to be destroyed by proteolytic enzymes, which would indicate that it is of proteid nature. Hemolysis is produced both *in vitro* and *in vivo* with all sorts of blood, but to very different degrees, thus resembling spider toxin. Locally bee poison causes necrosis, with marked hyperemia and edema. A 4500 gm. dog was killed by intravenous injection of 6 c.c. of a 1.5 per cent. solution of pure poison (Langer¹).

Immunity is undoubtedly possible, for bee-keepers frequently show a great decrease in susceptibility. On the other hand, abnormally great susceptibility is frequently seen, some cases of fatal poisoning having been observed.²

Ants also produce formic acid, a fact so well known that it has come to be considered that this is the source of their toxicity. Von Fürth, however, suggests the probability that ant poison, like that of the bees, owes its chief effects to other more complex, unknown poisons.

POISONS OF DERMAL GLANDS OF TOADS AND SALAMANDERS

It has been known for centuries that toads produce poisonous substances, Paré in 1575 having discoursed interestingly, if inaccurately, on this topic. Numerous studies have been made of these poisons, which are secreted by the dermal glands and therefore cannot be used for poisoning either prey or enemies (except those that feed upon them); the most extensive study being that of Faust.³ He isolated two constituents, apparently the same, in different species of toads; one, which he called *bufotalin*, is very active, resembling the digitalis group; the other, *bufonin*, is much less active. Bufonin is neutral in

¹ Arch. exp. Path. u. Pharm., 1896 (38), 381; Arch. internat. Pharmac. et Ther., 1899 (6), 181.

² Hospitalstidende, 1905, No. 27.

³ Arch. f. exp. Path. u. Pharm., 1902 (47), 279. Complete bibliography and review.

reaction, soluble in warm alcohol, but slightly in cold. Analysis indicates an empirical formula of $C_{17}H_{27}O$, which is probably but half the molecular formula. It probably is the cause of the milky appearance of the dermal secretion. Bufotalin seems to be $C_{34}H_{46}O_{10}$, is acid in reaction, soluble in chloroform and alcohol, but not in petroleum ether. Subcutaneous injection of 2.6 mg. bufotalin killed a dog (weighing 4 kg.) in four to five hours; given by mouth it causes much vomiting and diarrhea, so that large doses are not fatal. It causes much local irritation when applied to mucous membranes, but produces no marked changes at the site of injection. The effects on the circulation resemble in all respects those of the digitalis group; bufonin acting similarly but much weaker than bufotalin. Bufotalin seems to be derived from bufonin by oxidation, and the latter is quite similar to cholesterolin, apparently having the following formula: $HO-H_{26}C_{17}-C_{17}H_{26}-OH$.

Phisalix and Bertrand¹ have found poison in the blood of toads similar to that of the glands. The hemolytic property observed by Pugliese² may be due to the acidity of the dermal secretion. The poisons of different species seem to be quite the same in all (Faust).

Salamanders also produce poisonous secretions in their dermal glands, which have been studied especially by Faust,³ and earlier by Zalesky,⁴ who isolated an inorganic base which he named *samandarin*. Faust describes samandarin as first stimulating and then paralyzing the automatic centers in the medulla. The poison resembles the alkaloids, having the formula $C_{26}H_{40}N_2O$, and produces death in doses of 0.7–0.9 mg. per kilo (dogs) with respiratory failure. Immunization of rabbits was practically impossible. A second alkaloid, *samandaridin* ($C_{20}H_{31}NO$) is also present in even greater quantities than the samandarin, and differs only in being weaker.

Bert⁵ and also Dutartre⁶ have also described a digitalis-like poison in the secretion of the dermal glands of *frogs*.

It is evident that all of these poisons are quite distinct from the venoms, and from the true toxins, apparently being simple chemical compounds not related to the proteids and not capable of causing immunization. The same is true of *cantharidin*, which is, according to Meyer,⁷ an acid with the following formula:

¹ Arch. d. physiol. norm. et path., 1893 (5), 511.

² Archivio di farm. e terap., 1894 (2), 321; Arch. ital. de Biol., 1895 (22), 79.

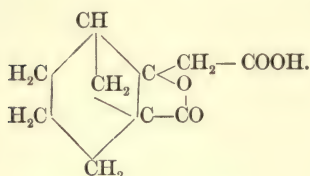
³ Arch. exper. Path. u. Pharm., 1898 (41), 229 (literature); 1900 (43), 84.

⁴ Hoppe-Seyler's Med. Chem. Untersuch., 1866, p. 85.

⁵ Compt. Rend. Soc. Biol., 1885, p. 524.

⁶ *Ibid.*, 1890, p. 199.

⁷ Lit. given by Faust, "Die tierischen Gifte," p. 210.

POISONOUS FISH ¹

There are numerous fish, especially in tropical waters, which defend themselves by injecting poisons into their enemies. This is accomplished by spines, to which are attached poison glands. Dunbar-Brunton ² has described two such fish (*Trachinus draco* and *Scorpaena scorpha*) of Mediterranean waters. Wounds by these spines cause in animals intense local irritation and edema and paralysis of the part, followed by gangrene about the site of the wound; in fatal poisoning death occurs in from one to sixteen hours, with general paralysis. The sufferings of persons so poisoned are said to be extreme, and death may occur either directly from the poison or later from sepsis following the local gangrene. Presumably this poison is not dissimilar to that of the snakes; it probably is not an alkaloid, as Dunbar-Brunton suggests. It affects chiefly the heart, according to Pohl. ³

Several other fish secrete poison in glands attached to long spines, one of the most poisonous being *Synanceia brachio*, which is known to have caused fatal intoxication in several instances. Only the *Muraenidae* seem capable of poisoning by biting; they have a well-developed poison apparatus on the gums, but nothing is known concerning the poisons they produce.

Many fish develop poisonous ptomaines remarkably soon after death, especially in tropical climates, so that a fish that is perfectly wholesome if eaten immediately after being caught may be very poisonous if kept but a few hours. There is a decided difference in fish of different varieties in this respect, so that some cannot be safely marketed.

There are also other fish whose bodies, even when perfectly fresh, contain very powerful poisons. Savtschenko, ⁴ in his elaborate atlas of the poisonous fish, describes a number of cases of poisoning by the famous "parrot fish" of Japan (*Tetrodon*), in which the poison seems to be developed and contained in the ovaries and eggs, and therefore the degree of toxicity varies

¹ Full discussion and literature given by Faust, "Tierische Gifte," p. 134.

² Lancet, 1896 (ii), 600.

³ Prager med. Woch., 1893 (18), 31.

⁴ "Atlas des Poissons Veneneux," St. Petersburg, 1886 (literature).

with the season of the year in which the fish is taken. Poisoning by these fish is very violent, the symptoms appearing very soon, and the cases are divided into two groups by Savtschenko, as the algid, or choleric form, and the gastro-intestinal type. The symptoms of the algid form appear almost immediately after eating the fish, and consist of pain in the stomach, with great fear and distress; soon diarrhea and vomiting set in, with cramps in the arms and legs; this terminates in collapse, coma, and death from either respiratory or cardiac paralysis. The entire course of the process may be but ten to twenty minutes, or it may be as many hours. On account of the localization of the poison in the eggs and ovaries not all persons who eat the fish are poisoned, and not all who are poisoned receive a fatal dose. In the gastro-intestinal form the symptoms appear later, consist chiefly of gastro-intestinal disturbances resembling more closely ptomain poisoning, and the prognosis is not so bad as in the algid form.

The pathological anatomy of this form of poisoning has not been carefully studied, but no characteristic or striking anatomical changes have been noted in the bodies examined. Tahara¹ has described a crystalline body, *tetrodonin*, and a toxic acid, *tetrodonic acid*, which are highly toxic; these were isolated from the ovaries of *Tetrodon*.²

In this connection may be mentioned the peculiar erysipelas-like lesions caused by bites of crabs, which indicates the formation of some toxic product by these crustaceans. Gilchrist³ obtained a history of bites or injuries by crabs in 323 of 329 cases of "erysipeloid."

EEL SERUM

In 1888⁴ Mosso studied the toxic properties of eel serum, which he found was extremely poisonous for experimental animals, 0.1 to 0.3 c.c. per kilo being fatal for rabbits and dogs in a few minutes if intravenously injected; introduced into the stomach it is not toxic. The poisonous principle he called *ichthyotoxin*. Death results from respiratory failure with large doses; small doses lead to cachexia and death after a few days. The coagulability of the blood is greatly reduced. Kossel⁵ found histological changes in the central nervous system in such animals, that resembled the lesions of tetanus. He succeeded

¹ Ref. in Maly's Jahresber., 1894 (24), 450.

² Arch. exp. Path. u. Pharm., 1890 (26), 401 and 453.

³ Jour. Cutaneous Diseases, November, 1904.

⁴ Arch. Ital. de Biol., 1888 (10), 141; 1889 (12), 229.

⁵ Berl. klin. Woch., 1898 (35), 152.

in securing an active antitoxin which neutralized the strongly hemolytic action of eel serum *in vitro*, and also prevented fatal effects in animals. Camus and Gley¹ have studied the physiological action of eel serum and found it strongly hemolytic, and also apparently neurotoxic. The toxicity is destroyed by heating to 58° for fifteen minutes. By immunization an antitoxic serum can be obtained which neutralizes the eel toxin completely. Tchistovitch² secured antitoxic serum, which acted also as a precipitin for eel serum. De Lisle³ found that eel serum does not act like an amboceptor, since after heating it cannot be reactivated with fresh serum, and it seems, therefore, to be different from snake serum in its structure.

¹ Arch. internat. d. Pharm., 1899 (5), 247.

² Ann. Inst. Pasteur, 1899 (13), 406.

³ Jour. of Med. Research, 1902 (8), 396.

CHAPTER IX

HEMOLYSIS AND SERUM CYTOTOXINS

CYTOTOXINS

JUST as precipitins can be obtained for proteids derived from other sources than bacterial cells, so also upon immunizing an animal against various types of cells other than bacteria, substances appear in its serum that exercise a destructive effect upon the type of cells injected. In other words, the reactions of animals to infection are not specially devised for combating bacteria and their products, but can be equally exerted against non-bacterial cells and their products. It may be stated as a general law that a certain degree of immunity, accompanied by the appearance of specific "antibodies" in the serum, may be obtained by injecting any sort of foreign cell or proteid substance into an animal; but that such immunity cannot be obtained unless the injected material is of a proteid nature or very closely related to the proteids, *e. g.*, enzymes and toxins. In the case of soluble proteids, as before mentioned, the antibodies show their effects by precipitating them, with agglutination of the particles into flocculi; in the case of cells, whether bacterial or tissue cells, the antibodies cause agglutination and loss or impairment of vitality. This injury may be manifested by loss of motion in motile cells (bacteria, spermatozoa, ciliated epithelium) or by solution of their contents (bacteriolysis, erythrocytolysis, leucocytolysis, etc.), or by cell death without marked morphological alterations (*B. typhosus*, spermatozoa). If we inject red corpuscles, leucocytes, spermatozoa, renal epithelium, or any other foreign cell, the reaction is, therefore, as specific as it is if we inject bacteria, and of exactly the same nature. Therefore, all that has been said previously concerning bactericidal substances and agglutinins can be transposed to apply to immunity against tissue cells. As a matter of fact, however, the transposition is generally made in the other direction, for red corpuscles are much easier cells to study than bacteria, because their laking gives prompt and readily recognized evidence that the toxic serum has brought about changes. Much of our knowledge of bactericidal serum has been obtained through studies of the mechanism of erythrocytolysis, the results

of which have then been applied to the subject of bacteriolysis. Both on this account, therefore, and because solution of red corpuscles is of itself an important process in many intoxications and diseases, the subject is of great theoretical and practical importance.

HEMOLYSIS¹ OR ERYTHROCYTOLYSIS

In hemolysis the essential phenomenon consists in the escape of the hemoglobin from the stroma of the corpuscles into the surrounding fluid. As it is not exactly known in what way the stroma holds the hemoglobin normally, whether purely physically or in part chemically, or whether the stroma consists of a spongioplasm or a sac-like membrane, or both, the ultimate processes that permit the escape of the hemoglobin are not finally solved. However, the agents by which the escape is brought about are well known and extensively studied, and they are found to be of extremely various natures. They may be roughly classified as: (1) known physical and chemical agents; (2) unknown constituents of blood-serum; (3) bacterial products; (4) certain vegetable poisons; (5) snake venoms.

HEMOLYSIS BY KNOWN CHEMICAL AND PHYSICAL AGENCIES

The Mechanism of Hemolysis.—If distilled water is added to corpuscles of any kind, osmotic changes are bound to occur, since within the cells are abundant salts, soluble in water, which will begin to diffuse outward in an attempt to establish osmotic equilibrium between the corpuscles and the surrounding fluid. Conversely, water enters the corpuscles at the same time, and accumulating there leads to swelling until such injury has been produced as permits the hemoglobin to escape and enter the surrounding fluid. Before this occurs the fluid is opaque because of the obstruction to light offered by the red cells, but on the completion of hemolysis the fluid becomes transparent. The stroma now settles to the bottom, while the hemoglobin diffuses into the fluid, making it red, but perfectly transparent. This process has long been known as the “laking” of blood, and is essentially the condition present in all forms of hemolysis. That the hemoglobin escapes only through injury of the stroma and not through simple osmotic diffusion is shown by the fact that if salt solution of the same concentration as normal serum is used instead of distilled water, no such escape of hemoglobin

¹Through usage this term has been limited to the solution of the red corpuscles, which is more accurately described by the term *erythrocytolysis*. For bibliography see Sachs, *Ergebnisse der Pathol.*, 1902 (7), 714.

occurs. As hemoglobin is perfectly soluble in the salt solution, it should pass out if it diffused as do the salts. Since there is no escape of hemoglobin in such a salt solution, it is evident either that the stroma is not permeable to hemoglobin, or else the hemoglobin is in some way attached to or combined with the stroma. Again, if the corpuscles are placed in a solution of salt more concentrated than their own fluids, water escapes and the corpuscles shrink; as no hemoglobin escapes with the water, it is evident that the stroma is not permeable to hemoglobin when intact. Because of the resemblance of the process of hemolysis to the rupture of plant cells with escape of their contents when they are placed in distilled water, it might be assumed that hemolysis is largely a physical matter, but there are many indications that chemical changes must be involved. For example, if a red corpuscle in an isotonic solution is cut into pieces, the hemoglobin does not escape, indicating that its structure is quite dissimilar to that of the simple vegetable cell, and that there is some union of stroma and of hemoglobin other than a physical union.¹

Repeated alternate freezing and thawing is another physical means of bringing on hemolysis. Heating to 62°–64° C. causes hemolysis of mammalian corpuscles; in cold-blooded animals this seems to occur at a slightly lower temperature.

Some chemical agents are capable of liberating hemoglobin, even when the corpuscles are in isotonic solutions. The ordinary salts of serum, of course, do not have this property, but ammonium salts are strongly hemolytic. The chemical agents that dissolve red corpuscles seem to be those that have the power of penetrating the stroma. Ammonium salts and urea penetrate the corpuscles freely and causes hemolysis. Sugar and NaCl seem not to penetrate the corpuscles, and therefore do not produce hemolysis. Of the permeating substances, there seem to be two types: one, like urea, does not produce hemolysis when in a solution of NaCl isotonic with the serum; the other, like ammonium chloride, is not prevented from producing hemolysis by the presence of NaCl.²

¹ Stewart (Jour. of Physiol., 1899 (24), 211) found that in hemolysis by physical means or under the influence of serums, there is no marked increase in the electrical conductivity, but hemolysis by saponin and by water causes an increase of conductivity, presumably because of the escape of electrolytes.

² Hamburger, in his book, "Osmotischer Druck und Ionenlehre," reviews exhaustively the physical chemistry of hemolysis. The following is his summary of the permeability of red corpuscles by various substances:

Organic Substances.—(a) *Impermeable* for sugars; namely, cane-sugar, dextrose, lactose, also arabit and mannit. (b) *Permeable* for alcohols, in inverse proportion to the number of hydroxyl groups that they contain; also for alde-

All these agents seem to effect hemolysis *by acting on the stroma*, for when the stroma of corpuscles hardened in formalin has its lecithin and cholesterin removed with ether, *saponin*, a powerfully hemolytic substance, seems to have no effect. The action of saponin and of many other hemolytic agents can be prevented by the presence of *cholesterin* in excess, suggesting that it is this constituent of the stroma that is affected.¹

The fact that chloroform, ether, bile salts, and amyl alcohol will cause laking is probably intimately connected with the fact that lecithin and cholesterin, important constituents of the stroma, are both soluble in these substances.² Arseniuretted hydrogen, when inhaled, causes intravascular hemolysis, and there are many other drugs and chemicals with the same property, among which may be mentioned nitrobenzol, nitroglycerin, and the nitrites, guaiacol, pyrogallol, acetanilid, and numerous aniline compounds. Probably the hemolysis produced by autolytic products belongs in this category.³ The bile acids and their salts will also produce hemolysis, as seen in jaundice. Sodium bicarbonate solutions of one or two per cent. are hemolytic for some varieties of corpuscles, but 0.1 per cent. Na_2CO_3 and NaHCO_3 do not cause hemolysis.

Leucocytes are dissolved by some of these agents, particularly the bile salts, although they are affected by no means so rapidly or so much as are the erythrocytes. There seems to be no relation between the erythrolytic and leucolytic powers of these substances. Water causes swelling, with solution of the granules in time, and the same is true of ammonium-chloride solutions.

HEMOLYSIS BY SERUM

Normal blood-serum of many animals causes hemolysis to greater or less degree when mixed with red corpuscles of another species of animal, and this property can be greatly increased by immunizing the animal with red corpuscles in the usual way.

hydes (except paraldehyde), ketones, ethers, esters, antipyrin, amides, urea, urethan, bile acids and their salts. (c) *Slightly permeable* for neutral amino acids (glycocoll, asparagin, etc.).

Inorganic substances, not including the salts of the fixed alkalies. (a) *Completely impermeable* for the cations Ca, Sr, Ba, Mg. (b) *Permeable* for NH_4 ions, for free acids and alkalies.

¹ Ransom, Deut. med. Woch., 1901 (27), 194; Kobert, "Saponinsubstanzen," Stuttgart, 1904; Abderhalden and La Count, Zeit. exp. Path. u. Ther., 1905 (2), 199. Noguchi (Univ. of Penn. Med. Bull., 1902 (15), 327) found lecithin without this property.

² See Koeppel, Pflüger's Arch., 1903 (99), 33; Peskind, Amer. Jour. Phys., 1904 (12), 184.

³ Concerning hemolysis by alcohols, ketones, etc., organic acids, and essences, see Vandevelde, Bull. Soc. chim. de Belgique, 1903 (19), 288.

This hemolysis occurs both in the test-tube and in the body, in the latter case causing severe anatomical changes or even death. In all respects *the mechanism of hemolysis by serum seems to be identical with that of bacteriolysis*. Two substances are concerned, one, the *amboceptor*, which resists heat and which is increased by immunizing; the other, *complement*, which is destroyed at 55° and which is present in normal serum. In this case the substances may be referred to as hemolytic amboceptors and hemolytic complements.¹

In spite of the availability of these particular cytolytic substances for study, very little has been learned of their exact nature and properties. It is known that amboceptor is combined with the red cells in a certain sense quantitatively, a certain amount being required to saturate a given amount of corpuscles so that they will all be hemolyzed when complement is added; and that this reaction is complete in less than fifteen minutes at 45° . What change this addition of amboceptor brings about in the corpuscles is unknown. It has also been shown that at 0° the affinity between the amboceptor and the corpuscle is greater than it is between amboceptor and complement, so that it is possible at this temperature to remove all the amboceptor from a serum by treating it with red corpuscles, and thus we can obtain complement free from amboceptor. This experiment also shows that the two bodies exist side by side in the serum without combining, and that combination occurs only after the amboceptor has become united to the erythrocyte.

The Amboceptor.—Amboceptor is, as a rule, destroyed by heating to 70° or higher. Its place of origin is unknown. Metchnikoff holds that it is derived chiefly from the leucocytes, in support of which view is the fact that leucocytes dissolve red corpuscles after ingesting them; however, other phagocytic cells have the same power, particularly endothelial cells, and it is an open question whether the intracellular digestion of engulfed cells is the same process as extracellular hemolysis; probably it is not, for there seem to be more disintegrative changes in intracellular digestion than in hemolysis. Quinan² found that the diffusible constituents of hemolytic serum played no rôle beyond that of maintaining osmotic pressure. He was unable, however, to localize the immune body in any of the proteid constituents. Kyes found that a combination of hemolytic amboceptor of venom and lecithin gave no biuret reaction. The amboceptors

¹ Bang and Forssmann (Hofmeister's Beitr., 1906 (8), 238) do not accept the prevailing view.

² Hofmeister's Beitr., 1904 (5), 95.

of normally hemolytic serum seem to be no different from those in immune serum, and amboceptors of one animal can combine with complement furnished by the serum of an entirely different animal. It is the amboceptor alone that gives the specific nature to the reaction, and, as is the case with all other immunizations, it is very difficult to secure antibodies by immunizing an animal with blood from another animal of its own species, *isohemolysins*; and impossible to secure antibodies for its own blood, *autohemolysins*.

Although Bordet and other French observers have claimed that the union between amboceptor and corpuscle is physical and not chemical, the evidence seems to point the other way.¹ Probably the union is with the stroma rather than with the hemoglobin, and the result of the union is to render the stroma permeable to the hemoglobin, or to separate the bonds that unite the hemoglobin to the stroma. Mathes² contends that red corpuscles cannot be dissolved by hemolytic serum or by pancreatic juice until after they have been killed; as heated serum does not kill them, this is presumably done by the complement. Corpuscles that have been killed can then be dissolved in their own serum. Levene³ tried to produce hemolytic serums by immunizing with different constituents of corpuscles, using—(1) pure crystalline hemoglobin; (2) proteids of the stroma soluble in salt solutions; (3) an extract with alcohol-ether; and (4) an extract in 1.5 per cent. sodium bicarbonate. Only the last gave positive results, and the serum was almost devoid of agglutinative properties. Injection with corpuscles that had been digested with trypsin gave about the same results as alkaline extracts; corpuscles digested by pepsin gave a much weaker serum; in neither was agglutination obtained. According to Bang and Forssmann⁴ ethereal extracts of red corpuscles give rise to production of hemolysins on immunization, and this "lysinogen" substance can be precipitated with acetone, is insoluble in alcohol, is not destroyed by boiling, and gives rise to no agglutinin. Ford and Halsey⁵ obtained serum with both lytic and agglutinative powers by injecting either the stroma or the laked blood free of stroma; results with pure hemoglobin

¹ Bang and Forssmann (Hofmeister's Beitr., 1906 (8), 238) suggest that the amboceptor merely renders the corpuscle permeable for the complement, perhaps through action on the lipoid membrane; the complement then acts directly upon some constituent of the corpuscle, without the amboceptor acting as a combining substance in any way.

² Münch. med. Woch., 1902 (49), 8.

³ Jour. Med. Research, 1904 (12), 191.

⁴ Hofmeister's Beitr., 1906 (8), 238.

⁵ Jour. Med. Research, 1904 (11), 403.

were indefinite. Stewart¹ obtained similar results by immunizing with corpuscles laked by physical means, by serums, or by saponin. According to Guerrini,² nucleoproteid obtained from dog's blood will give rise to specific hemolysins, and Beebe has found that nucleoproteids from visceral organs do not have this effect. Levene's alkaline extracts probably also contained nucleoproteids.

The Complement.—Hemolytic complement possesses the same properties as bacteriolytic complement, resembling enzymes to the extent that it is susceptible to heat and causes a disintegration of cells. The joint action of amboceptor and complement is strikingly like the activation of trypsinogen by trypsin, or of glycolytic enzyme by pancreatic extract. On the other hand, hemolysis by serum is quite different from the effect of trypsin on corpuscles, as trypsin completely disorganizes the hemoglobin and destroys the stroma, while in hemolysis the stroma and hemoglobin seem to be merely separated from one another but not chemically altered. Again, *hemolysin acts quantitatively*, although that may be due to a difference in the way the binding to the cell occurs, rather than in the method of action of the complement.

Probably complement is produced in leucocytes, and perhaps in many or all other cells, but removal of the spleen does not prevent either the presence of complement or the formation of immune bodies. Although the serum of one animal may complement the immune bodies in serum of several other varieties, and also produce lysis of many sorts of cells, there is probably not one complement that does all the complementing; Ehrlich and others have proved that one serum may contain several complements of slightly differing natures. It is also known that heat-resisting substances may act as complement, and Kyes has demonstrated that red corpuscles may contain within themselves a hemolytic complement, *endocomplement*. For cobra poison lecithin may act as complement, and it has been possible to isolate the lecithin-venom combination.³

Antibodies can be obtained for both complement and hemolytic amboceptor by immunizing against serum containing them, and in many serums *antihemolysins* exist normally. Against certain vegetable hemolysins this antihemolytic action is very strong (Kobert). Antihemolysins are generally anticomplements, but in a number of instances anti-amboceptors have been obtained.

¹ Amer. Jour. of Physiol., 1904 (11), 250.

² Riv. crit. di clin. med., 1903 (4), 561.

³ Kyes, Berl. klin. Woch., 1903 (40), 957.

Hemagglutinin.—Agglutination of red corpuscles occurs under the influence of immune serum as well as under the influence of some normal serums. In all respects the principles seem to be the same as those described for bacterial agglutination. Agglutination occurs at much lower temperatures than hemolysis, and also is not checked by heating the serum to 55° ; hence it is possible to observe hemagglutination independent of hemolysis. Serums may contain hemagglutinins and not be hemolytic; the reverse is also true. As agglutination occurs in corpuscles that have been fixed in formalin or sublimate, it is probably not the proteids that are affected, but some other of the ingredients of the stroma, of which lecithin and cholesterolin seem to be the chief.

Certain *vegetable poisons* also produce agglutination of red corpuscles, especially ricin, abrin, and crotin, and the fact that ricin has little or no hemolytic action shows the independence of the processes. *Snake venoms* contain agglutinins, destroyed by heating to 75° ; their agglutinating power being in inverse ratio to their hemolytic power. Corpuscles agglutinated by venoms may be again separated by potassium permanganate solutions.¹ Silicic acid and certain other *colloids* may act as agglutinins, their effects bearing a relation to the effects of electrical charges upon agglutination of bacteria or of colloids (*q. v.*).²

Agglutination of the corpuscles during life may be of some pathological importance, for such masses of agglutinated corpuscles may readily produce capillary thrombi and emboli, which, if wide-spread, may create much disturbance. Many bacteria produce substances that are agglutinative for human red corpuscles, among them being *B. typhosus*, *pyocyaneus*, and staphylococcus. Flexner³ has found in typhoid fever thrombi that seemed to be composed of agglutinated red corpuscles, almost free from fibrin and leucocytes. Probably many of the so-called "hyaline thrombi" found frequently in infectious diseases are really composed of agglutinated, partly hemolyzed red corpuscles (see "Thrombosis," Chap. xi).

HEMOLYSIS BY BACTERIA

Both pathogenic and non-pathogenic bacteria produce hemolytic substances that are excreted into the fluids in which they grow. During many infectious diseases marked hemolysis occurs,

¹ See Flexner, Univ. of Penn. Med. Bull., 1902 (15), 361 and 324.

² See Landsteiner and Jagic, Münch. med. Woch., 1904 (51), 1185.

³ Univ. of Penn. Med. Bull., 1902 (15), 324; Amer. Jour. Med. Sci., 1903 (126), 202.

especially in those diseases accompanied by septicemia. After death the hemoglobin of the blood goes into solution, and the resulting staining of the walls of the blood-vessels, and later of the tissues everywhere, is generally familiar. In the *post-mortem hemolysis* probably the putrefactive organisms are chiefly concerned, although it is marked a very short time after death in many cases of septicemia, particularly when the infecting organism is the streptococcus, and here probably the pathogenic organism is the chief cause of the hemolysis. The hemolytic action of bacteria can be studied both *in vitro* and *in vivo*. Among the best known are *tetanolysin*, *pyocyanolysin*, *typholysin*, *staphylolysin*, and *streptocolysin*, as they have been termed. Of these, the case of pyocyanolysin is questionable, because it has been described as resisting heat above the boiling-point, and Jordan¹ seems to have proved that the hemolysis is ascribable to the alkalinity that this organism produces in culture-media. Other bacterial hemolysins are, however, destroyed by heat at 70° C. for two hours; but they are altogether different from ordinary cellular hemolysins. G. Ruediger² shows the following differences between streptocolysin and the hemolysins of serum; streptocolysin is not destroyed at 65° C. for one-half hour, and therefore is different from complement. When destroyed by heating to a higher point, it cannot be reactivated by the addition of complement, thus differing from intermediary body. It is also different from intermediary body in that it does not combine with corpuscles at 0° C.; on the other hand, it does combine at 6° C., but does not exert any hemolytic effect until the mixture is raised to a higher temperature. This last observation indicates that the streptocolysin is similar in nature to the toxins, namely, a toxophore group and a haptophore group. In other words, streptocolysin is simply a toxin for red cells, and unites directly to the cell receptors without the intervention of any intermediary body. As a similar structure has been shown for staphylolysin and tetanolysin, it is probable that the *bacterial hemolysins are all merely toxins with a particular affinity for red cells*.³

Secondary hemolysis occurring in the infectious diseases is

¹ Jour. Medical Research, 1903 (10), 31.

² Jour. Amer. Med. Assoc., 1903 (41), 962.

³ Abbott and Gildersleeve (Jour. Med. Research, 1903 (10), 42) consider that the hemolysis observed with some bacterial cultures is simply proteolysis by the contained enzymes.

According to v. Eisler, (Wien. klin. Woch., 1906 (19), No. 23) normal horse serum contains two substances antagonizing tetanolysin and staphylolysin. One is cholesterin, the other is precipitated out with the serum globulin and is destroyed by peptic digestion.

probably to be explained largely by this hemolytic property of bacterial toxins. Hemoglobinuria may also be produced in the same way in some instances. Intravenous injections of filtrates of the saprophyte, *B. megatherium*, will produce hemoglobinuria in guinea-pigs, hence hemolysis is not an exclusive property of pathogenic bacteria.

HEMOLYSIS BY VEGETABLE POISONS

A number of plant poisons are strongly hemolytic, and some of them owe much of their toxicity to their effect on the erythrocytes. One group consists of the bodies often called "vegetable toxalbumins," because they seem to be proteids, and includes ricin, abrin, crotin, and robin. Of these, crotin and phallin are particularly actively hemolytic, while ricin, abrin, and robin are more marked by their agglutinating action, hemolysis being produced only by relatively large doses. Their effects vary greatly, however, according to the species of animals whose blood is used. They resemble the bacterial toxins in that immunity can be secured against them, and the immune serum will prevent their hemolytic action. Heating the toxalbumins to 65° or 70° does not destroy the hemolytic or agglutinating action except with phallin, but 100° does. The action of these substances is not like that of the enzymes, in that it is quantitative, a given amount acting on a given amount of corpuscles to which it is bound. Madsen and Walbum¹ observed that red corpuscles had the power of dissociating neutral mixtures of ricin and antiricin, the ricin entering the corpuscles from which it could be recovered.² (The general nature and other properties of these substances have been considered under the heading of "Phytotoxins," in the preceding chapter.)

Phallin, from *Amanita phalloides*, is considered by Kobert to be of albumose nature, a "toxalbumin," and its principal effects are due to its hemolytic action, which is said to be equal to or stronger than that of cyclamin (Kunkel). Ford³ found the hemolytic principle of *Amanita* to resemble the bacterial hemolysins in acting directly upon corpuscles without the presence of serum. It dissolves all varieties of erythrocytes, is inactivated at 65° for one-half hour, is not inhibited by cholesterolin and lecithin, but is inhibited by serum and by milk, and destroyed by digestive enzymes. Immune serum neutralizes this hemolytic property. Besides the hemolytic poison, phallin, there is a thermostable poison which is strongly toxic but not hemolytic.⁴

¹ Cent. f. Bakt., 1904 (36), 242.

² According to Pascucci (Hofmeister's Beitr., 1905 (7), 457), ricin combines directly with lecithin, the compound being strongly hemolytic.

³ Jour. Infect. Diseases, 1906 (3), 191.

⁴ See Ford, Jour. Exp. Med., 1906 (8), 437.

Saponin Group.—Another quite distinct group of vegetable hemolyzing agents consists of the "*saponin substances*."¹ These are a closely related group of *glucosides*, found in at least 46 different families of plants, and they are strong protoplasmic as well as hemolytic poisons. They differ altogether from the true toxins, being heat resistant, having no resemblance to proteids, and not giving rise to antibodies on immunization of animals.² The degree of their toxicity is not directly proportional to their hemolytic activity; they seem to injure chiefly the nerve-cells. Apparently hemolysis is brought about by action upon the lipoids of the red corpuscles, for addition of cholesterol to saponin prevents its hemolytic effect;³ lecithin does not have the same property.⁴ Both cholesterol and lecithin combine with saponin, the cholesterol compound being quite inert, whereas the lecithin compound is both hemolytic and toxic. Cholesterol is also soluble in sapotoxin. Normal serum seems to contain an anti-hemolysin for saponin, and therefore hemoglobinuria is not produced by all saponins on intravenous injection. Careful immunization leads to a slight increase in this antihemolytic action of the serum, possibly due to an increased formation of cholesterol (Kobert).

A study of the toxicity of the members of this group by Kobert⁵ shows that in general they have similar properties, but that minor differences exist between them. All cause hemolysis, some in dilution as great as 1 : 100,000. Some produce hemoglobinuria when injected intravenously, others do not. All paralyze the heart, but the injuries to the central nervous system are the chief cause of death. Marked local changes are produced at the site of injection, but the leucocytes are apparently not injured, although sterile suppuration is produced. There is a period of latency after intravenous injection of small doses—twenty-four hours or more—before the appearance of symptoms.

SAPOTOXIN is one of the most actively toxic and hemolytic products of *quillaja*.

¹ Complete literature on saponin given by Kobert, "Die Saponinsubstanzen," Stuttgart, 1904; also Kunkel, "Handbuch der Toxikologie," Jena, 1904.

² Saponins are characterized by their ready solubility in water and the foaming, soapy character possessed by the solution; hence their technical applications as soap bark, etc. Heated with dilute acids they split off sugar; also when acted on by glucoside-splitting enzymes (from spiders), according to Kobert. Saponin from *Quillaja* (soap-bark) has the formula $C_{19}H_{30}O_{10}$ (Stütz). Most are colloids, but some crystallize.

³ Ransom, Deut. med. Woch., 1901 (27), 194; Madsen and Noguchi, Cent. f. Bakt., 1905 (37), 367.

⁴ Noguchi, Univ. of Penn. Med. Bull., 1902 (15), 327.

⁵ Arch. exp. Path. u. Pharm., 1887 (23), 233.

CYCLAMIN is also a member of this group (derived from *Cyclamen*), and is said to be the most active of all as a hemolytic agent (Tufanow).

SOLANIN¹ is obtained from all parts of the potato plant, combined with malic acid; it is found particularly in young sprouts, but not in any considerable amounts from normal potatoes.² Its formula is unknown, but as it splits up into an alkaloid (*solanidin*) and sugar it is called a *glyco-alkaloid*. In its action it resembles the saponins, being a powerful protoplasmic poison, killing bacteria, and hemolyzing blood in very great dilutions.

A number of hemolytic poisons are obtained from poisonous mushrooms. Best known of these is:

HELVELLIC ACID, from *Helvella esculenta*, which has the empiric formula $C_{12}H_{20}O_7$.³ Intravenously injected it produces hemoglobinuria and icterus, with hemoglobin infarets in the kidneys (Bostroem⁴).

As will be seen, all of these last-mentioned vegetable hemolytic agents are essentially different from either the bacterial or serum hemolysins, or from the abrin, ricin, crotin, or robin group, in that they are of relatively simple chemical composition, and quite unlike proteids, enzymes, or toxins. The manner in which they cause hemolysis is unknown, but from their relation to saponin it is probable that, like it, they cause injury by combining with or dissolving the lipoids of the stroma of the corpuscles.

HEMOLYSIS BY VENOMS⁵

The laking of blood-corpuscles by venoms is of peculiar interest from the standpoint of Ehrlich's theory, since it has been demonstrated by Flexner and Noguchi⁶ that the hemolytic principle of the venoms is an amboceptor. As venom contains no complement this has to be furnished by the blood, and so in the case of venom poisoning the victim furnishes the complement that destroys its own corpuscles. The hemolytic amboceptors of venom seem to be secreted by the salivary glands of

¹ Literature, see Meyer and Schmiedeberg, Arch. f. exp. Path. und Pharm., 1895 (36), 361; Perles, *ibid.*, 1890 (26), 88.

² See Kunkel, "Handbuch der Toxikologie," p. 873.

³ Boehm and Külz, Arch. exp. Path. u. Pharm., 1885 (19), 403.

⁴ Deut. Arch. klin. Med., 1883 (32), 209.

⁵ General review of literature on the hemolytic properties of animal poisons given by Sachs, Biochem. Centralblatt, 1906 (5), 257.

⁶ Jour. Exper. Med., 1902 (6), 277; Univ. of Penn. Med. Bull., 1902 (14), 438; and 1902 (15), 345.

the reptiles from their blood, which contains almost identical amboceptors, differing chiefly in that they can combine only with the complement contained in snake blood, while the amboceptors of venom can combine with the complement of nearly all sorts of blood. Venoms from cobra, rattlesnake, moccasin, and copperhead possess in each variety intermediary bodies (amboceptors) that seem to be identical in nature, although they may vary in quantity. This explains the rather remarkable fact that serum of animals immunized against cobra poison, generally called *antivenin*, will neutralize the hemolytic and many of the other properties of the venom of rattlesnake, copperhead, and moccasin. Antivenin acts as an anti-intermediary body, and by occupying a haptophore group of the amboceptor, prevents its completing the union of complement and cell. In order of decreasing hemolytic power for mammalian corpuscles come venoms from cobra, water moccasin, copperhead, and rattlesnake. These venoms are also agglutinative for all corpuscles tried, and agglutination will occur at 0° C. Exposure for thirty minutes at 75°–80° C. destroys the agglutinating property. In general, the hemolytic power of the venoms for different sorts of corpuscles varies in inverse proportion to its agglutinative power. The hemolytic intermediary bodies are remarkably resistant to heat, suffering but slight loss of power at 100° C. Red corpuscles of the frog are not hemolyzed by venom, and those of *necturus* (mud puppy), but slightly, agreeing with the known resistance of cold-blooded animals to snake-bites.

The highly hemolytic cobra venom can combine with complements contained within the red corpuscles, *endocomplement*, and so produce hemolysis in the absence of serum complement. Kyes has shown that lecithin may be the constituent of red corpuscles that acts as the complement.

Eel serum is remarkably hemolytic, so much so that a quantity of 0.1 c.c. per kilogram of body weight will kill a rabbit or guinea-pig in three minutes when injected intravenously. Heating at 54° C. for fifteen minutes destroys the hemolytic action, and, unlike ordinary serum hemolysins the addition of complement does not restore its activity. Animals can be immunized against this serum. Introduced into the stomach in ordinary quantities eel serum is not toxic. It can be dried and redissolved without losing its activity, but acids and alkalis readily destroy it. Mosso, who first discovered the toxicity of eel serum, called the unknown active principle *ichthyotoxin* (see preceding chapter).

HEMOLYSIS IN DISEASE

During health there is always going on a certain amount of destruction of red corpuscles that have outlived their usefulness; hence in disease we may have to deal with either an alteration in the normal processes of blood destruction or the introduction of entirely new processes. Although the place and manner of normal red corpuscle destruction is not completely known, yet it seems probable that there is relatively little hemolysis within the circulating blood. When a red corpuscle becomes damaged, it seems to become more susceptible to phagocytosis, and it is then picked out of the blood, chiefly by the endothelial cells of the sinuses of the spleen, hemolymph glands, and bone-marrow. Within these cells it apparently undergoes hemolysis. Eventually, the resulting pigment is split up by the liver, the non-ferruginous portion forming the bile-pigments, while the iron seems to be mostly withheld to be worked over into new hemoglobin. (See "Pigmentation," Chap. xvi.) Whenever during disease red corpuscles are more rapidly injured than they are under normal conditions, these processes of normal hemolysis are exaggerated and we not only find the phagocytic cells of the spleen and glands packed with corpuscles, but endothelial cells elsewhere, and also leucocytes, take on the hemolytic function. At the same time there results an excessive production of bile-pigment from the destroyed red corpuscles, which has an etiological relation to the so-called "hemato-hepatogenous" jaundice. If hemolysis is very excessive, the blood pigment accumulates in other organs than the liver and spleen. When at one time over one-sixtieth part of the hemoglobin of the blood is in solution in the plasma, it may escape in the urine, producing hemoglobinuria.

The hemolysis of the acute febrile diseases is readily explained by the demonstrable hemolytic property of the products of the organisms that cause them, such as streptocolysin, staphylolysin, etc. Perhaps at the same time products of altered metabolism may also play a part, but it does not seem probable from experimental results that the thermic condition *per se* has much effect. In malaria, although the parasites enter and destroy the corpuscles in which they live, yet this alone does not account for all the blood destruction of the disease, for the amount of anemia is quite without relation to the number of parasites to be found. There is good reason to believe that the plasmodia produce hemolytic substances that are discharged into the serum.¹ In the primary anemias hemolysis seems to be the

¹ Regnault, Rev. d. Méd., 1903 (23), 729.

essential process, although the agents involved are at present unknown. Absorption of hemolytic products of intestinal putrefaction or infection has always come in for much suspicion, without ever becoming completely established. Here also the hemolysis seems to take place in the endothelial cells rather than in the vessels. In such a disease as pernicious anemia there is much reason to assume that defective or abnormal hematogenesis is an important factor. Probably the anemia of nephritis is the result of hemolytic action of the retained products of metabolism, in which connection the hemolytic properties of ammonium compounds may be recalled. In some diseases associated with anemia it has been found that the blood-serum of the patient is distinctly *isohemolytic*, although *isoagglutination* seems to be more frequent. The fluids that can be obtained from cancers have been found to be hemolytic, while antihemolysin has been found in ascitic and pleural effusions.

In many forms of poisoning hemolysis is a prominent feature; in some it seems to be the chief effect of the poison, *e. g.*, potassium chlorate and arseniuretted hydrogen. In severe extensive burns there may occur hemolysis, and hemoglobinuria may also result. The remarkable "paroxysmal hemoglobinuria" is at present without satisfactory explanation as to the cause of the hemolysis except that during the paroxysm the blood is hemolytic. The hemoglobinemia of "blackwater fever" has been the cause of much discussion as to whether the malarial parasite or the quinine is the cause, with a divided opinion resulting, although, undoubtedly, cases do occur in malaria without administration of quinine. After removal of the spleen hemolysis by the hemolymph glands exceeds that of the primitive spleen, causing an excessive destruction of red corpuscles (Warthin¹). This suggests that the spleen may normally dispose of some hemolytic agent which acts either by stimulating phagocytosis or by so altering the red cells that they are particularly susceptible to phagocytosis.

Pathological Anatomy.—The lesions produced in the organs of animals injected with hemolytic agents are usually pronounced and quite characteristic. There is often a subcutaneous edema, which is usually blood-stained, and similar fluid may be present in the serous cavities. The fat is yellowish, and the muscles are darker in color than is normal. The spleen is usually much swollen, soft, friable, and very dark in color. The liver is usually swollen and mottled with red areas in a yellow background. The renal cortex is dark in color, even

¹ Jour. Med. Research, 1902 (7), 435.

chocolate-colored, and the pyramids are comparatively light; hemoglobin is frequently present in the urine. In the lungs are often found hemorrhages or areas resembling small infarcts. The blood may be thin and even distinctly transparent. Microscopically the red corpuscles are found in all conditions of degeneration, and often fused together. In the liver, besides patches of congestion, fatty changes are present if the animal lives long enough. Large phagocytic cells packed with red corpuscles are abundant in the spleen and lymph-glands, as well as diffuse accumulations of the blood-cells, which are often fused; and much pigment is also present, both free and in the cells. Pigment also accumulates in the renal epithelium, which often shows much disintegration; congestion is prominent, and hemorrhages into both interstitial tissue and glomerules are frequent. Some of the lesions are due to the hemolysis, and some to the associated agglutination of corpuscles, which form hyaline thrombi.

Agglutination of corpuscles in the vessels during life is undoubtedly of much pathologic importance, for such masses of agglutinated corpuscles may produce extensive formation of capillary thrombi and emboli, from which serious results may be produced. (See "Hyaline Thrombi," Chap. xi.) Many bacteria produce substances that are agglutinative for human red corpuscles, among them being such important disease-producers as typhoid, pyocyaneus, and staphylococcus. Flexner¹ has found in typhoid fever thrombi that seemed to be composed of agglutinated red corpuscles, practically free from fibrin and leucocytes. Probably many of the "hyaline thrombi" frequently found in infectious diseases are really composed of agglutinated, partly hemolyzed red corpuscles. Pearce² has found that agglutinative serum when injected into dogs causes widespread necrosis in the liver, which is followed by proliferation of connective tissue and the production of changes resembling cirrhosis.

CYTOLYSIS IN GENERAL

Not the same degree of success has been obtained in immunizing against other tissue elements as with the erythrocytes. Immune serum can readily be obtained against cells that can be secured quite free from other cells, such as spermatozoa, ciliated epithelium, and leucocytes, but even then the immunity is not specific.

¹ Univ. of Penn. Med. Bull., 1902 (15), 324; Amer. Jour. Med. Sci., 1903 (126), 202.

² Jour. Exp. Med., 1906 (8), 64; Jour. Med. Research, 1906 (14), 541.

Much less is it specific when ground-up organs are used for immunizing, as is the case in the experimental production of *nephrolysins*, *hepatolysins*, etc., for at the same time antibodies are secured for not only the typical parenchyma cells, but also for endothelium, stroma cells, red and white corpuscles, and blood plasma. As a consequence, the early expectations that by this process of immunization against specific cells great progress could be made in our knowledge of physiology, by selectively throwing out of function an organ through the simple process of injecting an antiserum, have been disappointed. Equally little progress has been made in the treatment of malignant growths by the same method. The immune serums usually obtained do, to a certain extent, injure the specific organ, but they also usually injure other organs nearly as much or perhaps more; furthermore they generally contain hemolytic toxins, even if the tissues used in immunizing are free from blood, and, as we have seen, hemolytic poisons may cause serious tissue destruction.¹

Beebe² has introduced a method of immunization that may yield better results. On the assumption that the nucleoproteids are the most characteristic constituent of the cells, he isolated them from different organs, and claims to have secured serums by immunizing with these nucleoproteids that were altogether specifically toxic for the type of cells from which the nucleoproteids were obtained; *e. g.*, immunizing with liver nucleoproteids yielded serum destroying liver cells and no others.

In view of the present uncertain state of the subject, and the very questionable value of much of the work so far done, the consideration of the various cytolsins or cytotoxins may be dismissed by briefly referring to a few of the most important results.

Leucocytolytic Serum.³—This may be obtained either by immunizing with leucocytes obtained from exudates or from the blood, or by using emulsions of lymph-glands. Specific leucocytolytic serum agglutinates leucocytes and produces observable morphologic changes, in the way of solution of the cytoplasm and cessation of ameboid movements. Of the leucocytes, the large granular cells seem most affected and the lymphocytes least. When injected into the peritoneal cavity such serum causes an apparent initial leucopenia, and later a decided

¹ See Sata, Ziegler's Beitr., 1906 (39), 1.

² Jour. Exp. Med., 1905 (7), 733.

³ Literature, see Flexner, Univ. of Penn. Med. Bull., 1902 (15), 287; Ricketts, Trans. Chicago Path. Soc., 1902 (5), 178; Christian, Deut. Arch. klin. Med., 1904 (80), 333.

leucocytosis in the peritoneal fluid. Corresponding with this, if bacteria are injected at the same time as the serum, resistance is found decreased, but later it is much increased. Such serum also contains anticomplement, according to Wassermann, indicating that the injected leucocytes contain complement. Leucocytotoxin obtained by immunizing against lymphatic tissue is very thermolabile, being destroyed by 55° C. for thirty minutes, and the serum can be only partially reactivated by the use of fresh serum.

Endotheliolytic Serum.—Every attempt at immunizing an animal with any sort of fixed tissue must of necessity involve the injection of endothelial cells as well as the cells specific to the tissue studied. Therefore, it is possible that cytotoxic serum so obtained will contain endothelial toxins and so complicate any results of *intra vitam* experiments. There is every reason to believe that endotheliolytic substances are produced in this way. Ricketts found that serum of animals immunized against lymph-glands was toxic to endothelial cells, which was indicated by hemorrhages at the point of injection, and marked desquamation of endothelium when the injection was made into a serous cavity. In snake-venom poisoning the extensive hemorrhages are also due to an endotheliolytic principle, called by Flexner *hemorrhagin*.

Lymphatolytic Serum.—This serum has been studied by Ricketts and by Flexner, who immunized animals with lymph-glands. As might be expected from the structure of the injected glands, the resulting serum contained endotheliotoxin, leucocytotoxin, hemolysin, hemagglutinin, leucocyto-agglutinin, and precipitins. When injected into animals, this serum has a marked effect upon the spleen and lymph-glands, producing great enlargement and congestion of these structures. The bone-marrow is also somewhat affected, and when marrow is used in immunizing, the *myelotoxic* serum produces marked proliferative changes in the lymph-glands as well as in the marrow.

Nephrolytic Serum.—It has been claimed that if a kidney is destroyed by ligating its vessels or ureter, the remaining kidney develops serious degenerative changes, which are not present if one kidney is entirely removed. This has been attributed to the development of nephrotoxic substances produced in reaction to the absorption of the injured renal tissue that has been left in the body. Other methods of renal injury have been thought to produce similar effects, and serum of animals with kidney disease was said to injure the kidneys of normal animals. Upon this basis it has been thought possible

to explain the progressive nature of the chronic nephritides as the result of nephrotoxins produced through the absorption of the injured cells, which nephrotoxins injure still other renal cells. Such a process, however, involves the production of cell toxins in an animal that are toxic for its own cells, that is, *autocyto-toxins*; and as it has so far been practically impossible to produce autolysins of other sorts, it is not altogether probable that the kidney is an exception. Furthermore, Pearce¹ was unable to produce isonephrotoxins, and could not corroborate the statements as to the changes said to have been found in the remaining kidney after ligating the vessels of its mate. He did obtain an active heteronephrolysin, but also found that immunization with liver produced nearly as actively nephrolytic serum as did immunization with kidney.

Neurolytic Serum.—Even as highly specialized cells as those of the nervous tissue seem to produce a reaction with the formation of immune bodies. Perhaps the most positive results are those of Ricketts and Rothstein,² who found that serum of rabbits immunized against the brains or cords of guinea-pigs was highly toxic when injected into the vessels of guinea-pigs, causing death with various symptoms only explainable on the assumption of nervous lesions. Microscopically, the ganglion-cells showed marked changes in those animals that survived the injection long enough. All the results so far obtained have been with heterogeneous serum. Venoms, particularly that of cobra, possess strong neurolytic substances that are the chief toxic agents in most of the venoms (rattlesnake venom excepted).

Thyrolitic Serum.—There are but few reports on this serum, but that of Portis³ indicates that after removal of all hemolysis as a factor there do occur changes, in the nature of excessive absorption of colloid, and proliferation after the order of that seen in thyroid regeneration. However, the clinical picture of thyroidectomy was not produced in any case, and the anatomic changes were not great. By immunizing against nucleoproteids derived from thyroid tissue, Beebe⁴ has secured an antiserum which seems to have some effect upon diseased thyroids (exophthalmic goiter). MacCallum⁵ could not get a specific serum for parathyroid tissue.

Numerous reports may be found indicating attempts, with varying success, to obtain serums toxic for other tissues. Among

¹ Univ. of Penn. Med. Bull., 1903 (16), 217.

² Trans. Chicago Path. Soc., 1903 (5), 207.

³ Jour. Infectious Diseases, 1904 (1), 127.

⁴ Jour. Amer. Med. Assoc., 1906 (46), 484. ⁵ Med. News, 1903 (83), 820.

them may be mentioned *epitheliolysin* (for ciliated epithelium), *spermatotoxin*, *hepatolysin*, *cardiolysin*, *splenolysin*, and *syncytiolysin*. Attempts at the production of immune serum with adrenal by Abbott¹ resulted only in a serum with great hemolytic power, but with no particular effect on the adrenal. In general it can be said that it has *not* been found possible in this way to throw out of function one particular organ, with or without involvement of other structures. The most suggestive results have been obtained by Beebe,² who has used nucleoproteids of specific organs in immunizing.

The principles involved in all these experiments are the same, and the results are in no instance altogether satisfactory; therefore no further consideration of these special cytotoxic serums will be made here, the reader being referred to a complete résumé and bibliography of the subject by Sachs³ for details.

¹ Jour. Med. Research, 1903 (9), 329.

² Jour. Exp. Med., 1905 (7), 733.

³ Biochemisches Centralblatt, 1903 (1), 573; *et seq.*; also see Sata, Ziegler's Beitr., 1906 (39), 1.

CHAPTER X

INFLAMMATION

ALTHOUGH morphological alterations are prominent features of the reaction of the tissues to local injury and infection, yet at the bottom the processes of inflammation are brought about by and result in chemical alterations. The causes of inflammation are in nearly all cases chemically active substances, but for the most part their nature is too little known to permit of speculation as to what chemical characteristic or characteristics a substance must possess to exhibit the power of causing an inflammatory reaction. Even in the case of inflammation due to mechanical, thermal, and electrical injuries, it seems probable that most of the features of the inflammatory reaction are brought about by the action of chemical substances produced by alterations in the tissue constituents at the point of injury.

The essential features of inflammation, namely, local hyperemia and related vascular disturbances, exudation of plasma, migration of leucocytes and their phagocytic action, all may be caused by the action of chemical substances upon the vessels and leucocytes. *Active hyperemia* in the case of inflammation is due to stimulation of the vasodilator nerves or paralysis of the vasoconstrictors, or direct paralysis of the muscular fibers of the arterioles; these may result from mechanical, thermal, or electrical stimuli, but in local infection the cause is usually chemical products of bacterial growth or of tissue disintegration. The *escape of blood plasma* (inflammatory edema) appears to depend upon a number of factors (discussed more fully under "Edema," Chap. xii) of which the most important seem to be: (1) injury to the capillary walls, produced largely by the chemical causes or products of the inflammation; (2) increased osmotic pressure in the tissues, due to increased or abnormal formation of crystalloidal substances with high osmotic pressure from large molecular compounds, many of which are colloids (proteids) without appreciable osmotic pressure. By far the most characteristic feature of inflammation, however, is the *behavior of the leucocytes*—their increase in number in the blood, their migration from the vessels and accumulation about the point of injury, and their engulfing and destroying various

solid particles, such as bacteria and degenerating tissue elements. These processes, which seem to indicate something approaching independent volition on the part of the leucocytes, may, however, be well explained by application of known laws of chemistry and physics, without passing into the realms of the metaphysical. This will be attempted under the heading of:

AMEBOID MOTION AND PHAGOCYTOSIS

The accumulation of leucocytes at a given point in the body indicates that some means of communication must exist between this point and the leucocytes in the circulating blood. No direct communication by the nervous system or other structural method existing, the only possible explanation is that the communication is through the fluids of the body, and depends upon changes in their chemical composition or physical condition. As the latter generally depends upon the former, the communication is considered to be accomplished by chemical agencies, and called *chemotaxis*.

CHEMOTAXIS

Changes in the chemical composition of a fluid have been shown frequently to affect the motion of living organisms suspended in it. One of the earliest observations was that of Engelmann,¹ who noticed that *Bacterium termo* suspended in water tended to accumulate about a bubble of oxygen in the water. Pfeffer² discovered that the spermatozoids of certain ferns were attracted powerfully by very dilute solutions of malic acid, which is contained in the female sperm cell, indicating that the migration of the sperm cells in the proper direction depends on a chemical communication, and he proposed the term chemotaxis for this phenomenon. Strong solutions of malic acid, on the other hand, repelled spermatozoids. Cane-sugar was found to attract the spermatozoids of a certain foliaceous moss. In the case of the malic acid, it seems to be the anion that produces the effect, since salts of malic acid have exactly the same property.

Stahl's³ experiment with a large jelly-like plasmodium (*Aethalium septicum*) growing on bark in wet places, has become classical. He found that if the plasmodium was placed on a moist surface, and near by was placed a drop of an infusion of oak bark, the organism moved by the process of protoplasmic stream-

¹ Botanische Zeitung, 1881 (39), 441.

² Untersuch. aus dem Bot. Institut in Tübingen, 1881-1888, Bd. 1 und 2.

³ Botanische Zeitung, 1884 (42), 145 and 161.

ing toward and into the infusion. If a piece of oak bark was placed in the water, plasmodial arms were stretched out to it and the piece of bark was soon completely surrounded by the organism. These movements were found to occur in any direction, even exactly against the force of gravity. Other substances, as acids or strong solutions of salt or sugar, were found to cause the plasmodium to move away from them, although when sufficiently dilute they exerted an attraction. A plasmodium might, however, move into a strong sugar solution if kept with a scanty supply of moisture for some time, and after it had lived in such a strong solution (2 per cent.) for some time, a weaker solution or pure water was as injurious as the concentrated sugar solution previously had been.

Temperature was also found to exert a marked *thermotactic* effect. If a plasmodium was placed on a filter-paper, one end of which was in water at 7°, and the other in water at 30°, it would move toward the warmer end.

The Theory of Tropisms.—Ciliated protozoa, which can move about freely in water, show very distinct reactions to stimuli of all sorts. The first step in their change of direction of movement is considered by many observers¹ to be an orientation of the organism until it is headed in the axis along which it is to move. This is ascribed by Loeb² to the existence of a certain degree of equality of irritability of symmetrical parts of the body. The stimulant, whether it be rays of light, or diffusing chemicals, or heat-waves, moves along definite lines, and the organism receives at first unequal stimuli on symmetrical parts of the body, unless the axis of the organism is parallel to the lines of motion of the stimulant. As long as the stimulant acts on symmetrical parts of the body unequally, these parts will react unequally until at length the body is swung into a position where the stimulation is equal, when it will stay in this position and move along a line parallel to the line taken by the stimulant. Not only protozoa, but much higher forms, including some vertebrates, are believed to react in this way to stimuli—*e. g.*, the maintenance by fish of a position heading up stream. The above constitutes the so-called "*theory of tropisms*," and we have such reactions to stimuli of all sorts, not only *chemotropism* and *thermotropism*, but also *heliotropism* (reaction to light); *geotropism* (to gravity), *electropism* (to electricity), *thigmotropism* (reaction to contact), etc.

¹ Jennings does not accept this view, but attributes the results to processes of "trial and error."

² Comparative Physiology of the Brain, New York, 1900, p. 7.

The work done upon tropisms applies particularly to ciliated, freely motile organisms, and interests us less in connection with leucocytes than do the observations on such forms as *Amœba*.¹ In passing may be mentioned the *thigmotaxis* or *thigmotropism* (reaction to mechanical stimuli) shown by spermatozoa, which explains their apparently difficult feat of advancing in opposition to the cilia of the epithelium lining the female generative tract. It may also be noted that the nature of reactions of organisms to various stimuli is not constant for even the same organism. Copepods. (minute crustacea) may be negatively heliotropic in the day and go away from the bright surface of the water, whereas at night the same animals are positively heliotropic and swarm to the surface, illuminated brightly by a lantern. Variations in heliotropism may, in some cases, be explained as due to chemical changes that occur in the organism, which explanation is made more probable by J. Loeb's experiments, which show that change in composition in the fluid in which animals are suspended may cause a complete reversal in their reaction to a constant stimulus.² Motile bacteria seem to behave much like ciliated protozoa in their reaction to stimuli.

CHEMOTAXIS OF LEUCOCYTES

That leucocytes come to the site of an infection because of chemical substances produced by bacteria at this point, that is to say, through chemotaxis, was first clearly pointed out by Leber³ in 1879, who likened the attraction of such substances for leucocytes to the effect of malic acid upon spermatozooids as shown by Pfeffer. He found that in keratitis leucocytes invaded the avascular cornea from the distant vessels, not in an irregular manner, but all moved directly toward the point of infection, where they collected. As dead cultures of staphylococci produced a similar, although less marked, accumulation of leucocytes, he sought the chemotactic substance in their bodies, and isolated a crystalline, heat-resisting substance, *phlogosin*, which attracted leucocytes in animal tissues. He also observed that capillary tubes filled with phlogosin or with staphylococci were soon invaded by masses of leucocytes.

Since Leber's experiments, many other investigations have

¹ For full details see Jennings (Publication No. 16, Carnegie Institute, Washington, 1904); also J. Loeb, "Studies in General Physiology."

² Barratt (Zeit f. allg. Physiol., 1904 (4), 87), however, was unable to demonstrate that quantities of acids and alkalies just sufficient to kill paramecium produced any change in the reaction of their protoplasm great enough to be detected by stains or by indicators, although it is well known that much smaller quantities exert marked chemotactic effects.

³ Fortschritte der Med., 1888 (6), 460.

been made showing that chemical substances of many different origins other than bacterial exert a chemotactic influence on leucocytes. Some substances are indifferent in effect, most are positive, while some are believed to repel leucocytes; *i. e.*, are negatively chemotactic.

Negative Chemotaxis.—Probably the substances that repel leucocytes are few in number; Kanthack, indeed, doubted the existence of really negative chemotactic action upon leucocytes. Verigo¹ also considers that as yet no actual negative chemotaxis has been satisfactorily demonstrated; but, by analogy with the effects of chemicals on ameba, ciliata, and plasmodial forms, which all show a decided negative chemotaxis under certain influences, it would seem most probable that leucocytes also should be repelled as well as attracted by chemicals.²

Non-bacterial Chemotactic Substances.—One of the earliest significant studies of the effects of non-bacterial substances upon chemotaxis was made by Massart and Bordet,³ who showed that products of the disintegration of leucocytes and other cells had a strong positive chemotactic influence. They also corroborated the statement of Vaillard and Vincent that *lactic acid* is an actively repellant substance, for they found that tubes containing a pyocyaneus culture, which ordinarily become filled with leucocytes rapidly, did not become invaded at all if lactic acid was also added in a strength of 1:500, although leucocytes did enter when the dilution was 1:1000.

Gabritchevsky⁴ studied the chemical influence of a large number of substances on leucocytes and divided them into three groups: I. Substances exerting "negative chemotaxis," including those that attracted only a few leucocytes.⁵ II. Substances with "indifferent chemotaxis," which attracted moderate numbers of leucocytes. III. Substances with positive chemotaxis. If we correct the groupings made by Gabritchevsky we have the following classification:

¹ Arch. d. Méd. exper., 1901 (13), 585.

² Salomonsen's observation (Festskrift ved indvielsen af Statens Serum Institut, Copenhagen, 1902, Art. XII), that ciliated infusoria when killed show a strong negative effect on other ciliates, is of much interest, particularly as it seems to be the opposite of the positively chemotactic effect of dead upon living leucocytes. The negative reaction of different ciliata was specific for their own kind quantitatively, but not qualitatively.

³ Ann. d. l' Inst. Pasteur, 1891 (5), 417.

⁴ Ann. d. l' Inst. Pasteur, 1890 (4), 346.

⁵ Evidently these substances were not all negatively chemotactic, but were relatively slightly chemotactic or indifferent; yet in the literature generally these experiments have been cited as indicating a negative chemotactic influence of the substances studied.

- I. Substances negatively chemotactic or indifferent :
 - (a) Concentrated solutions of sodium and potassium salts;
 - (b) Lactic acid in all concentrations ; (c) quinine (0.5 per cent.) ; (d) alcohol (10 per cent.) ; (e) chloroform in watery solution ; (f) jequirity (2 per cent., passed through Chamberland filter) ; (g) glycerine (10 per cent. to 1 per cent.) ; (h) bile ; (i) *B. cholerae gallinarium*.
- II. Substances with feeble chemotaxis :
 - (a) Distilled water ; (b) dilute solutions of sodium and potassium salts (1-0.1 per cent.) ; (c) phenol ; (d) antipyrin ; (e) phloridzin ; (f) papayotin (in frog) ; (g) glycogen ; (h) peptone ; (i) bouillon ; (j) blood and aqueous humor ; (k) carmine.
- III. Substances with strong positive chemotaxis :
 - (a) Papayotin (in rabbits) ; (b) sterilized living cultures of bacteria, whether pathogenic or non-pathogenic.

These results can only be considered as suggestive and not as accurate findings, in view of other contradictory results. Buchner¹ obtained from the *pneumobacillus* of Friedlander, a proteid which exerted a strong chemotactic influence, thus showing the chemical nature of the attraction of leucocytes by bacteria, and he isolated other similar proteids from other bacteria. He also obtained a "glutin-casein" from grain which was related chemically to the bacterial proteids, and which was equally chemotactic. The metabolic products of bacteria, however, he found to be negatively chemotactic. Alkali albuminate and hemi-albumose were strongly positive, but peptone was not. Glycocoll, and leucin were found to be chemotactic, but urea, ammonium urate, skatol, tyrosin, and trimethylamin were not. It was also observed that if the positively chemotactic substances were injected subcutaneously, they produced general as well as local leucocytosis.

v. Sicherer² found that chemotaxis of leucocytes may be observed outside the body. If a tube containing positively chemotactic substances (dead beer-yeast cells and dead staphylococci were the strongest) is placed with one end in a leucocyte-containing exudate, the leucocytes pass up into it against gravity.

Bloch³ demonstrated that carbol-glycerine extracts made from each of the different viscera and tissues exerted a positive chemotaxis, discrediting the statements of Goldscheider and

¹ Berl. klin. Wochenschr., 1890 (27), 1084.

² Cent. f. Bakt., 1899 (26), 360.

³ Cent. f. allg. Path., 1896 (7), 785.

Jacob that only extracts of hematogenous tissues showed positive chemotaxis. Egg-albumen, gelatine, albumen-peptone, and alkali albuminate were also positive, carbohydrates feebly so, and fat not at all. Metallic copper, iron, mercury, and their salts have also been found to be chemotactic substances, but it is very probable that they act in part through destroying the tissues in their vicinity, which give rise to decomposition-products having a positive effect. Adler,¹ however, found that bichloride of mercury as dilute as 1 : 3000 caused more leucocytic invasion of a piece of saturated elder pith than did even cultures of pyogenic bacteria.²

Metchnikoff observed that leucocytes might, after a time, be attracted toward substances that at first seemed to repel them. If the blood is full of toxins, the subcutaneous introduction of bacteria does not lead to a local accumulation of leucocytes, presumably because the difference in chemotaxis between the blood and the tissue fluids is not great enough to cause emigration; in this connection should be mentioned Pfeffer's observation that *B. termo* in a peptone solution will not migrate toward another stronger peptone solution unless the latter is at least five times as strong as the former.

Relation of Cell Types to Migration.—Of the leucocytes, the most actively affected by chemotaxis is the polymorphonuclear variety, but not all substances affect each variety of leucocyte in the same way; for example, infections with most animal parasites result in both local and general increase in the eosinophilous forms, and similar effects have been obtained by the injection of extracts of animal parasites. *Lymphocytes* are much less active, presumably because they contain less of the mobile cytoplasm and consist chiefly of the structurally fixed nuclear substance. Undoubtedly many of the cells in so-called lymphocytic accumulations seen in certain conditions, such as tuberculosis, are not lymphocytes from the blood, but are newly divided cells of the tissue.³ The experimental evidence concerning lymphocytic emigration is very contradictory. Fauconnet⁴ has found that tuberculin injections cause in man general increase in leucocytes, but only of the polymorphonuclear form. Long-continued intoxication of animals, however, may result in lymphocytic increase, but local introduction of the toxin leads to accumulation of polymorphonuclear cells and not lymphocytes.

¹ Festschr. for A. Jacobi, 1900, New York.

² Concerning the effects of iodine and iodides upon the leucocytes, see Heinz, Virchow's Arch., 1899 (155), 44.

³ See résumé by Pappenheim, Folia Hematol., 1905 (2), 815.

⁴ Deut. Arch. klin. Med., 1904 (82), 167.

Particularly significant is the experiment of Reekzeh,¹ who found that in lymphatic leukemia, with the lymphocytes greatly exceeding the polymorphonuclear forms in the blood, the pus from an acne pustule or from cantharides blisters contains practically no lymphocytes, but is composed of the usual polynuclear forms. Wolff,² however, claims that tetanus and diphtheria toxin produce lymphocytosis in experimental animals. Wlassow and Sepp³ state that lymphocytes are not capable of ameboid movement or phagocytosis in the body, although after heating to 44° they may become motile for a short time.

Experiments on the nature of the leucocytes attracted by different chemotactic agents have been made by Borissow⁴ and Adler.⁵ Both agree in stating that none of the substances tested shows any special affinity for any single type of leucocytes. Usually the polymorphonuclear cells in exudates far exceeded their proportion in the circulating blood. *Tissue cells* were found by Adler to migrate far into blocks of elder pith, apparently rather later than the leucocytes. As they showed changes of form indicating ameboid motions he considers their migration to be an active process. The existence of the polymorphonuclear forms in the pith seems to be very transient.

The position taken by the young blood-vessels and cells in granulation tissue, at right angles to the surface, possibly also depends on chemotaxis determining the direction in which the new cells shall proliferate.

Thermotaxis of Leucocytes.—Heat seems to affect leucocytes much as it does ameba, moderate temperatures being positively thermotactic. Mendelssohn⁶ states that the thermotaxis is most pronounced at a temperature of 36°–39° C. (97°–102° F.), but is still marked as low as 20° C. Temperatures higher than 39° C. (102° F.) do not seem to attract them. Wlassow and Sepp⁷ state that motility of leucocytes is increased by warming to 40° C., and that temperature of 42°–46° C. causes the movements to become very irregular, with feeble power of contraction. Lymphocytes are not motile at ordinary temperature, but at 44° they begin to move, and once motile, they continue their motion when cooled as low as 35°; this motility is considered to be entirely abnormal and only the result of degenerative changes.

Temperature probably plays but a minor part in attracting

¹ Zeit. f. klin. Med., 1903 (50), 51.

² Berl. klin. Woch., 1904 (41), 1273.

³ Virchow's Arch., 1904 (176), 185.

⁴ Ziegler's Beiträge, 1894 (16), 432.

⁵ Festschrift f. A. Jacobi, New York, 1900.

⁶ Roussky Vrach, 1903.

⁷ Virchow's Archiv, 1904 (176), 185.

leucocytes in pathological processes, however. The local heat of an inflamed area is due chiefly to the accumulation of blood in the part, and would not influence the leucocytes to migrate from the still warmer blood into the tissues. By increasing motility, however, the temperature of fever may favor migration and phagocytosis, and local application of heat to inflamed areas may induce local leucocytic accumulation. In burns the duration of the period of excessive temperature is usually too brief to account for the attraction of leucocytes that results; this accumulation is undoubtedly due to the products of the resulting cell degenerations.

The influence of light, mechanical stimulation, and gravity upon leucocytes seems not to have been studied. The phagocytosis of insoluble non-nutritive particles has been ascribed to *tactile stimulation*, but the details of the operation of such stimuli are unknown, and the entire question of tactile stimulation is unsettled. In experiments with elder pith it has been observed that leucocytes penetrate to the center, even when the pith contains only physiological salt solution. As Adler remarks, it is difficult to explain such migration as due to tactile stimuli; but, on the other hand, no other explanation has been offered.

PHAGOCYTOSIS

The engulfing of bacteria, cells, tissue products, etc., by leucocytes seems to be but an extension of the phenomenon of chemotaxis. When the substance toward which the leucocyte is drawn is small enough, the leucocyte simply continues its motion until it has flowed entirely about the particle. Later the particle becomes, as a rule, more or less altered within the cell, unless it is a perfectly insoluble substance, such as a bit of coal-dust. This action upon the engulfed object is undoubtedly due to the action of intracellular enzymes.¹ Protozoa take their food into a specialized digesting vacuole which has been shown by Le Dantec² (in *Stentor*, *Paramœcium*, and some other varieties) to contain a strongly acid fluid. Miss Greenwood³ has also demonstrated acid in several forms of protozoa, which is formed under stimulation of injected particles, whether nutritious or not. Mouton⁴ has been able to extract from the bodies of protozoa (rhizopods) a feebly proteolytic enzyme.

¹ See Opie, Jour. Exp. Med., 1906 (8), 410.

² Ann. d. l'Inst. Pasteur, 1890 (4), 776.

³ Jour. of Physiol., 1894 (16), 441.

⁴ C. R. Acad. des Sciences, 1901 (133), 244.

This "*amibodiastase*," as he calls it, is active in alkaline, and faintly acid media, and digests colon bacilli that have been killed by heat, but not living bacilli. This last fact is highly suggestive in connection with the important question of whether leucocytes engulf and destroy virulent bacteria or only those that have been previously injured by the tissue fluid. It was impossible to secure either invertase or lipase in extracts of protozoa. Whether bacteria are digested in leucocytes by the same enzymes that digest the leucocytes themselves after they are killed (*i. e.*, the autolytic ferments), or by some specialized enzyme, is not known. Metchnikoff, however, has noted the localized production of acid in the cytoplasm of leucocytes of the larva of *Triton taeniatus*. The eventual excretion of the remains of the bacteria or other foreign bodies by the phagocytes is ascribed by Rhumbler to changes in the composition in the particles through digestion, so that they have a greater surface affinity for the surrounding fluids than for the protoplasm of the cell.

Phagocytosis cannot be readily ascribed to chemotaxis, however, in the case of phagocytosis of perfectly insoluble, chemically inert particles, such as coal-dust. The leucocytes seem to take up foreign bodies without reference to their nutritive value, absorbing India-ink granules and bacteria impartially when they are injected together, and loading themselves so full of carmine granules that they cannot take up bacteria subsequently injected. It is possible that foreign bodies first become coated with a layer of altered proteid which then leads to phagocytosis, but there is no sufficient evidence for this surmise.

Not only leucocytes but tissue cells are capable of moving and performing phagocytosis when properly stimulated, and apparently all or nearly all fixed cells may act as phagocytes under some conditions. Their power of independent movement is much less than their phagocytic power. Endothelial cells are particularly active in phagocytosis, as also are the new mesodermal cells produced in inflammation. Apparently they obey the same laws as the leucocytes, and not only take up bacteria, but also fragments of cells and tissues, red corpuscles, and even intact leucocytes and other cells. Brodie¹ considers that phagocytosis by endothelial cells in lymph-glands is the natural end of the leucocytes, and red corpuscles seem to have a similar fate.

Phagocytosis is usually accomplished solely by the cytoplasm of the cells, the nuclei maintaining a passive rôle; but, according to Detre and Selli,² the phagocytosis of particles of lecithin

¹ Jour. of Anat. and Physiol., 1901 (35), 142.

² Berl. klin. Woch., 1905 (42), 940.

is accomplished by the nuclei, which seem to have a specific affinity for this substance.

Giant-cell formation may also be considered as the result of chemotaxis, the cells moving toward the attracting particle, and when the particle is larger than the cells they spread out upon its surface, their cytoplasm flowing together because of altered surface tension. The peripheral disposition of the nuclei probably depends on the fact that in ameboid motion the nucleus of the cell plays an entirely passive rôle, being dragged along by the cytoplasm, and hence it is located most remotely from the attracting particle. Digestion of materials taken into a giant-cell seems to go on as in the individual cells that compose it.¹

Influence of the Serum on Phagocytosis (Opson-ins).²—Phagocytosis of bacteria by leucocytes seems not to be merely a reaction between the leucocytes and the bacteria, as has been assumed by Metchnikoff and his school. Wright and Douglas³ have demonstrated that certain substances in the blood-serum are necessary to prepare the bacteria for phagocytosis, these substances being termed by them "*opsonins*." If leucocytes are washed free from serum with salt solution and let stand in a test-tube with such bacteria as *Streptococcus pyogenes*, *Staphylococcus pyogenes*, *B. typhosus*, *B. coli*, *B. tuberculosis*, and various other organisms, no phagocytosis occurs. If, however, some serum from a normal or an immunized animal is added to the mixture, active phagocytosis soon takes place. This opsonin is susceptible to heat, for if the bacteria are let stand with serum that has been previously heated to 60° for ten minutes, and then placed with the leucocytes, no phagocytosis occurs, but if unheated serum is used, the bacteria will be taken up by the leucocytes. These observations have been corroborated and extended by Hektoen and Ruediger.⁴ The opsonin acts upon the bacteria rather than upon the leucocytes. Certain salts were found to reduce considerably the degree of opsonic action by acting upon the opsonin itself. What changes the opsonins produce in the bacteria that makes them capable of attack by the leucocytes is unknown. The effect of negatively chemotactic substances (*i. e.*, substances preventing chemotaxis) depends upon their destroying the opsonin, according to the results obtained by Hektoen.⁵

¹ See Faber, Jour. of Path. and Bact., 1893 (1), 349.

² See also Immunity against Bacteria, Chap. vi.

³ Proc. Royal Soc., 1903 (72), 357; 1904 (73), 128.

⁴ Jour. of Infectious Diseases, 1905 (2), 128.

⁵ Complete résumé in the Jour. Amer. Med. Assoc., 1906 (46), 1407.

Results of Phagocytosis.—After phagocytosis has been accomplished, the fate of the engulfed object depends upon its nature. If digestible by the intracellular enzymes it is soon destroyed, but in the case of engulfed living cells, it seems probable that they must be first killed—they form no exception to the rule that living protoplasm cannot be digested. This brings forward the question of so much importance in the problems of immunity: Do living bacteria enter phagocytes, or are they first killed by extracellular agencies before they can be taken up? At the present time it seems to be positively established that leucocytes do take up bacteria which are still viable, and which may either grow inside the leucocyte or may be destroyed by intracellular processes.¹ On the other hand, leucocytes do not take up extremely virulent bacteria, and hence the question as to the relative importance played by the leucocyte and by the body fluids is still undetermined. It is probable that phagocytosis by fixed tissue-cells is of much less importance in checking bacterial growth than is phagocytosis by leucocytes. Thus Ruediger's experiments showed that emulsions of organs, with the exception of bone-marrow, do not destroy streptococci which are readily destroyed by leucocytes.

Indigestible substances may remain in cells, particularly in fixed tissue cells, for very long periods, if the substances are chemically inert. The leucocytes seem to transfer the indigestible particles which they have engulfed to other tissues, particularly to the lymph-glands; this is probably accomplished by phagocytosis of the laden leucocytes by the macrophages of the lymph sinuses, but how the insoluble particles are later transferred to the gland stroma or perilymphangial tissues, where they are chiefly found in such conditions as anthracosis, etc., is quite unknown.

THEORIES OF CHEMOTAXIS AND PHAGOCYTOSIS

On the assumption that leucocytes obey the same laws in their motions as do the amebæ, studies of the latter and of other forms of protozoa have furnished most of the ideas, hypotheses, and theories of the forces involved in leucocytic activities. The structural relation of the leucocyte to the ameba is striking, although, by no means complete; the relation of their activities is even closer. Each is a microscopic, independent, unicellular organism, moving freely in all directions by means of pseudopodia and protoplasmic streaming, taking other smaller bodies into its substance and digesting them, reacting similarly to like

¹ See Ruediger, Jour. Amer. Med. Assoc., 1905 (44), 198.

stimuli, and containing similarly a nucleus and many granules. The differentiation of the protoplasm of the ameba into a clear outer ectosarc and an inner granular endosarc is perhaps an important difference, but so far as the two forms of cells have been studied, the effect of this difference in structure does not seem to have been considered. That the unicellular protozoa, devoid of any central nervous system, and without any apparent co-ordinating mechanism, seem able to move about in a purposeful way, going toward food supplies and away from injurious agencies, toward or away from light, heat, and chemicals, has long attracted the interest of physiologists, particularly as in these single-celled organisms we may look for the simplest conditions of existence and the most elementary life processes. It seems absurd to imagine that a *paramœcium* goes toward a dilute acid because it "likes it," that an ameba rejects a piece of glass because it "does not taste good," as we explain similar manifestations in higher forms; furthermore, it has been shown by Verworn that minute enucleated fragments of protozoan cells react to stimuli just as does the entire cell, and, therefore, it seems that the only possible explanation of movements in protozoa must be a direct reaction of the stimulated part to the stimulus. The nature of the stimulus and the nature of the stimulated substance must determine the nature of the resulting reaction, and most of the observations so far made suggest that these reactions can be explained according to the known laws of the physics of fluids. An ameba, or a leucocyte, may be looked upon as a drop of a colloidal solution, surrounded by a delicate surface layer which is more or less readily permeable to solvents and to substances in solution, and suspended in a fluid of quite different composition.

Surface Tension.—Such a drop of fluid suspended in another different fluid obeys well-known laws of physics. The particles of each fluid are all under the influence of a very considerable force, called the cohesion pressure, which tends to draw them together closely. Within the drop each particle is subjected to this force alike from all sides, so that the forces neutralize one another, and each particle is as free as if there were no cohesion pressure. But the particles on the surface are subjected to unequal pressure, for that of the fluid outside the drop is different from that inside, and so the pressure on the surface particles is equal to the difference of the cohesion pressure of the two fluids; this constitutes the surface tension. It is this tension that pulls in upon the surface continually, causing it to tend always to reduce the free surface to a minimum, which condition exists perfectly in the sphere. The amount of cohesion affinity is very different in different fluids, and therefore some have a high surface tension and some a low. When a substance dissolves in another the surface tension is a resultant of the sur-

face tension of the two substances, and hence the surface tension of a liquid may be raised or lowered by dissolving various substances in it.

ARTIFICIAL IMITATIONS OF AMEBOID MOVEMENT

Imagining a drop of fluid suspended in water—let it be a drop of protoplasm, or oil, or mercury; the drop owes its tendency to assume a spherical shape to the surface tension, which is pulling the free surface toward the center and acting with the same force on all sides. The result is that the drop is surrounded by what amounts to an elastic, well-stretched membrane, similar to the condition of a thin rubber bag distended with fluid. If at any point in the surface the tension is lessened, while elsewhere it remains the same, of necessity the wall will bulge at this point, the contents will flow into the new space so offered, and the rest of the wall will contract; hence the drop moves toward the point of lowered surface tension. Conversely, if the tension is increased in one place, the wall at this point will contract with greater force than elsewhere, driving the contents toward the less resistant part of the surface, and the drop will move away from the point of increased tension. The resemblance of these changes of form and the type of motion produced to ameboid movement, is apparent, and much experimenting has been done to determine how far the processes of motion as shown by amebæ and leucocytes can be reproduced by fluid drops under various conditions of experiment, and to ascertain if such ameboid movement of living cells can be entirely explained by the laws of surface tension.

Gad,¹ in 1878, pointed out the resemblance to ameboid motion of the changes in shape observed in drops of rancid oils in weak alkaline solution. These changes in shape are due to the formation of soaps which lower the surface tension of the drop in places, so that the fluid flows toward these places and produces pseudopodium-like projections.

G. Quincke² later ascribed the contractions and other movements of ameba to alterations of the surface tension of the living substance in relation to that of the surrounding medium, believing the substances responsible for the alterations to be albuminous soaps.

Bütschli³ found that drops of "foam structure" made by mixing rancid oil and potassium carbonate solution show "*protoplasmic streaming*" when placed in glycerine, and that

¹ DuBois Reymond's Arch. f. Physiol., 1878, p. 181.

² Wiedmann's Annalen, 1888 (35), 580.

³ "Protoplasm," translation by Minchin, London, 1894.

they exhibit positive chemotaxis toward soap solution and other chemicals, the motion being accompanied by current formation in the drops. The "pseudopodia," formed by the drops also show currents rushing along their axes and returning by way of the surface. Heat leads to increased activity of motion. The motions were ascribed by Bütschli to the bursting of some of the superficial globules of the foam, which then spread over the surface of the drops, lowering its surface tension at the point of contact. He believed that ameboid motion, likewise, depended upon rupture of surface globules of protoplasm, for the "foam structure" of which he has been the leading advocate.

Bernstein,¹ basing his work on some observations of Paal-zow, observed that a completely inorganic substance, a drop of quicksilver, could be made to imitate ameboid motion under the influence of chemical changes. If near a drop of quicksilver in a nitric acid solution a crystal of potassium dichromate is placed, as soon as the yellow color made by diffusion of the dichromate reaches the drop, the quicksilver begins to show motion and advances toward the crystal. This movement is due to local oxidation of the surface mercury, which lowers the tension on that side of the drop, toward which the mercury then flows. If the crystal is removed, the drop follows, often flowing about it as if to take it in, but soon again withdrawing when the acid dissolves away the oxide formed on the surface, only to return again later. All these movements, which may be very life-like, are readily explained by changes in surface tension that take place under the influence of the bichromate and the acid, and are unquestionably referable to surface phenomena.

Artificial Amebæ.—By far the most suggestive experiments on the simulation of ameboid activity by non-living substances are those of Rhumbler (1898) in his great work, "*Physikalische Analyse von Lebenserscheinungen der Zelle.*"² On the assumption that the living protoplasm was but a more or less tenacious fluid, following the simple physical laws of fluids, especially in relation to its surface tension, he devised a number of experiments to determine the correctness of these views. An ameba may be regarded as such a mass of viscid fluid, in a medium in which it is nearly or quite insoluble; it is also constantly undergoing chemical changes within itself, and taking substances from or secreting them into the surrounding

¹ Pflüger's Arch., 1900 (80), 628.

² Arch. f. Entwicklungsmechanik, 1898 (7), 103.

water. To reproduce partly these conditions a drop of clove oil is placed in a mixture of glycerine and alcohol; the alcohol and clove oil are miscible, the glycerine merely retarding the diffusion.¹ Such a drop of oil will move about, changing its form and sending out pseudopodia much as an ameba does. These movements are undoubtedly due to changes in the surface tension brought about by the irregular mixing of the alcohol and the clove oil. The effect of chemotaxis upon an ameba can likewise be imitated with such an "artificial ameba." If some stronger alcohol is carefully introduced into the fluid near the drop, the surface tension on that side will be lowered, and the drop will flow in that direction. The effect of chemical changes within the drop upon its motion may be demonstrated similarly by injecting a little alcohol into the substance of the drop near one edge—the drop will send out a pseudopodium on that side, and perhaps flow along in the direction of the pseudopodium. We can imagine that metabolic changes in the body of an ameba may account for many of its seemingly purposeless movements by altering surface tension in some part of its circumference. Thermotaxis, the effect of heat in modifying or impelling ameboid motion, may be equally well demonstrated in such an "artificial ameba," the drop being "positively thermotactic," and flowing rapidly toward a heated point in the solution, because heat lowers the surface tension.

Even as highly specialized a process as the taking of food may be closely simulated experimentally. Amebæ seem to possess the faculty of selecting substances that are suitable for their food, crawling over particles of sand, wood, etc., and rejecting them when they are pushed against or into the surface of the ameba, which, however, readily takes up bacteria, diatoms, algæ, etc., digests them, and later throws out the undigested particles. If there is any property of the ameba that suggests voluntary action, it seems to be exhibited in the choice of its food, although this is not so well developed a selective process as might be expected, for amebæ will take up many harmful objects, and they may be made to fill themselves so full of useless substances that they cannot take up food. However, a drop of chloroform in water, which makes a good artificial ameba, if "fed" with various substances, will refuse some and take in others in a surprisingly life-like manner. Pieces of glass or of wood placed in contact with the drop, exert no influence; if pushed into the substance of the drop, they carry

¹ The details of these experiments are as given briefly by Jennings, *Jour. of Applied Microscopy*, 1902 (5), 1597.

the surface ahead, and on being released they are thrown out with some force. If a piece of shellac, paraffin, styrax, or Canada balsam be brought in contact with the surface of the drop, however, the drop flows around it immediately, and takes it within its substance, where it is soon dissolved. Even more strikingly like phagocytosis and intracellular digestion, however, is the result of a similar experiment with a piece of glass covered with shellac; the chloroform "ameba" takes it up as readily as it does the shellac alone, but after all the coating is dissolved away the piece of glass is then cast out of the drop. The resemblance to the engulfing, digestion, and excreting of indigestible particles of bacteria, etc., by amebæ, is so striking that it seems impossible that there can be any fundamental differences in the two processes. It will also be noticed that the drop takes in only what it can dissolve and rejects what it cannot.

One of the most remarkable actions of the amebæ which seems almost certainly the result of voluntary action is this: Oftentimes in feeding, an ameba gets hold of a suitable material which is in the form of a long thread, much too long for the ameba to surround. It then proceeds to coil up the thread within its body, by stretching a slight distance along the thread, bending over, and forming a bend in the thread, and by repeating the process it crowds the thread into a neat coil within its body, where it can be digested. The process is done so systematically and with such evident adoption of the means at hand to the desired end, that it seems as if it must be an adaptation of the ameba to circumstances, the result of long experience or of heredity. That an artificial ameba can perform the same maneuvers seems hardly credible, but it is readily done with almost no difference in detail. If the chloroform drop is given a long fine thread of shellac, it proceeds to bend the thread in the middle, and to send pseudopodia out along the thread to pull it into the drop, coiling it up inside as the chloroform softens the substance of the thread, until it is all contained within the drop, provided, of course, that it is not too long (a thread six times as long as the chloroform drop may be taken in completely). The bending and coiling of the thread in this experiment is entirely in accord with the known laws and phenomena of surface tension.

Fully as striking an ameboid action as the coiling up of a thread too long to be taken in, is the building, by some of the protozoa closely related to the ameba (*Diffugia*) of a shell which the animal seems to form by cementing together grains

of sand, or diatom shells, or other suitable particles. The particles are united so closely and fitted together so well that they are almost perfectly free from crevices. Even this process is accurately imitated in Rhumbler's experiments. If a drop of oil is mixed with fine grains of quartz sand, and dropped into 70 per cent. alcohol, the grains are thrown out to the surface, where they adhere to the surface of the drop and to one another exactly as do the particles in a diatom shell. So well fitted are the particles that the artificial shell may remain intact for months, and resemble the natural shell indistinguishably.

**RELATION OF THE ABOVE EXPERIMENTS TO THE PHENOMENA
EXHIBITED BY LEUCOCYTES IN INFLAMMATION**

The experiments cited indicate strongly, to say the least, that amebæ, and presumably leucocytes, react to stimuli of various kinds, chiefly through the effect of these stimuli upon surface tension. The stimuli may come from within the cell, being in this case the result of changes in composition brought about by metabolic processes; such chemical products alter the tension of the surface nearest their point of origin, causing what appears to be spontaneous motion. Stimuli acting from without may be chemical, thermal, electrical, or mechanical, but in any event they act as stimuli to motion through their effect upon surface tension; if they decrease the surface tension the cell goes toward them; if they increase the tension, the cell moves away. The behavior of leucocytes in inflammation may be explained on these purely physical grounds very satisfactorily, as follows:

At the point of cell injury or of infection, substances are produced that exert positive chemotaxis, as can be shown by experiments both outside and inside the body; these substances are chemotactic because they influence the surface tension of the leucocytes, and since with most if not all the products of cell disintegration the effect is to lower surface tension, the chemotactic effect is positive. As the chemotactic substances are produced, they diffuse through the tissues until they reach the walls of a capillary, through which they begin to pass, presumably most rapidly through the thinnest parts of the wall, the "stomata" and intercellular substance. The leucocytes passing along in the bore of the capillary will be touched by the chemotactic substances most on the side from which the substances diffuse; the surface tension will be lowered on this side, causing the formation of pseudopodia and motion in this direction. When the leucocytes come in contact with the wall, their surfaces, because saturated with the chemotactic substances,

will have a tension much the same as that of the cells of the capillary wall, which are likewise saturated with the same substances, and the two surfaces will tend to cling to one another; explaining the phenomenon of adhesion of leucocytes to the capillary wall, when, according to the usual description, "the leucocytes behave as if either they or the capillary wall had become sticky." Surface tension of the leucocytes will be least nearest the points where the most chemotactic substances are entering the capillary, namely, the stomata; hence the pseudopodia will form in this direction and flow through the openings, the rest of the cytoplasm flowing after and dragging the nucleus along in an apparently passive manner. Since it is the cytoplasm that seems to be chiefly affected in these processes, the nucleus appearing to be rendered inert by its relatively dense and fixed structure, the leucocytes with most cytoplasm are most active in emigration, while those with the least, the lymphocytes, are affected relatively little or not at all.

Once through the vessel wall, the motion continues in the same manner, toward the side from which the chemotactic matter comes, just as the mercury drop flows toward the crystal of potassium dichromate, or the drop of oil flows toward the alcohol. If the leucocyte meets a substance that lowers its surface tension sufficiently, it will flow around the object and enclose it, just as the chloroform drop flows about the piece of shellac or balsam; this constitutes phagocytosis. The motion of the leucocyte will continue in a forward direction until one of several possible things happens: (a) The leucocyte may reach a point where the chemotactic substances are so thoroughly diffused that the effects on its surface are the same on all sides; there will then be no tendency to move in any direction. (b) It may reach a material that exerts a marked positive influence upon it, causing much lowering of the surface tension, but which is so large that the cytoplasm flowing along its surface cannot surround it; other leucocytes will experience the same change, their cytoplasm will fuse together because of the equal lowering of their surface tension, and soon we get a mass of leucocytes with fused cytoplasm surrounding the object, forming a "foreign body giant-cell." (c) The leucocyte may reach a place where the concentration of the chemicals is so great that chemical changes are produced in its cytoplasm. If these changes are of a coagulative nature, the surface of the cell will be stiffened so that it cannot migrate further; if of a solvent nature, the leucocyte is destroyed. (d) It may reach the margin of an area where the preceding leucocytes have become coagu-

lated or otherwise rendered immobile, so that they block its path, while it is held fixed by the attraction on this side. (*c* and *d* explain the formation of solid leucocytic walls about areas of inflammation, and the frequent absence of leucocytes within the central necrotic areas.) (*e*) The formation of chemotactic substances may cease because the substance causing the inflammation has been used up, or because the bacteria have been destroyed, or from any of the causes that terminate inflammation. Those leucocytes still advancing will reach a point where there is as much chemotactic substance behind as in front—they will then stop advancing. As the fluids exuded in the central portion continue to dilute the chemotactic substances and wash them out, there will soon be less chemotactic substance in the center of the inflamed area than there is farther out, hence the leucocytes will move away from the center toward the periphery, following the chemotactic substances back into the blood-vessel and the lymph-stream. These are the conditions that exist at the close of the inflammatory process, which results in the dispersion of the leucocytes.

General leucocytosis can be explained equally well on the same grounds. Chemotactic substances from the area of inflammation enter the blood-stream, and so, in a very dilute form, pass through the bone-marrow. The chemotaxis in the blood will be greater than that of the marrow, and the leucocytes will move toward and into the blood. As long as the blood contains more chemotactic substances than the marrow, leucocytosis will increase, to stop when the amount in blood and marrow is alike or when there is less in the blood than in the marrow.

Behavior of Tissue-cells and Formation of Giant-cells.—The free cells of the tissues involved in inflammation can, of course, obey the same influences as the leucocytes, and apparently do so in so far as they are not checked by structural impediments to flowing motion; *i. e.*, the more closely a cell is related to a simple drop of fluid protoplasm, the more closely does it resemble in the simplicity of its reactions the “artificial ameba.” Cells with much cytoplasm are best fitted to move freely, as a rule, and hence we see chiefly the large endothelial cells of the lymph sinuses and the serous cavities, and the large hyaline and granular cells of the blood acting as phagocytes, for phagocytosis is no different from ameboid motion which continues about a particle until it is surrounded; likewise we see the “epithelioid” cells with their abundant cytoplasm fusing together to form giant-cells. (Note that such giant-cells are formed particularly in conditions in which the epithelioid cell is

more abundant than is the leucocyte, *e. g.*, tuberculosis and other chronic inflammations. The cells that fuse about an infected catgut ligature are the leucocytes, for they are most abundant in such a place.) A good illustration, also, is the giant-cell formed by fusing of leucocytes about blastomyces in minute abscesses in the epithelium in blastomycetic dermatitis; the epithelial cells cannot flow or coalesce well because of their abundance of stiff keratin and their specialized cell-wall, and hence do not participate; the leucocytes are individually too small to surround the fungus cells, and hence they flow about them in the abscess exactly as they will do experimentally in a test-tube or in a guinea-pig's abdomen (Hektoen). The formation of giant-cells is, on this ground, but an amplification of ameboid movement and phagocytosis. The fusing of the individual cells is due to the lowering of their surface tension by the materials diffusing from the body which is to be absorbed, until the surface of each cell becomes alike, when the surface tension at the point where each cell is in contact becomes zero and the cytoplasm runs together.

Objections to the above Hypothesis.—Physical explanations of ameboid movement seem to fit very perfectly the known facts concerning the actions of leucocytes. There arise but a few difficulties in applying these laws to leucocytic action; one is the phagocytosis of chemically inert bodies, such as coal particles, tattooing materials, stone dust, etc. We know that amebæ also may take up such inert materials, although they generally refuse them, and it is believed that the particles exert some local injury to the cell wall that leads to an alteration in its tension. Amebæ seem also sometimes to excrete a sticky substance over their surfaces or over the foreign matter that is to be engulfed, which excretion seems to be the result of surface stimulation. Possibly leucocytes do the same. We must bear in mind, however, that the protoplasmic cells have much greater possibilities for action than the "artificial ameba," since within the protoplasm countless chemical changes are going on which must cause continual alteration in surface tension; it is quite possible that mere mechanical action may alter chemical action at the point of contact, so that the injuring particle may become surrounded through local liquefaction of the protoplasm.

With the ameba, unfortunately, the explanation of all its activities by purely physical analogies is apparently not so successful. Although simple pseudopodia may be produced experimentally, and their formation explained readily on the surface tension basis, yet we find many forms of pseudopodia in

the great family of amebæ. Some of them are branching, some are fixed in extension, some have a stiff elastic axis. It would also be difficult to explain cilia as produced by changes in surface tension, yet we find in some protozoa that pseudopodia may take on the persistence and action of cilia, and that cilia may seem to change into pseudopodia. Jennings has made a most extended study of the relations of the "Behavior of Lower Organisms"¹ to the physical theories of ameboid motion, and is unable to corroborate the claim that the processes that go on in "artificial amebæ" exactly reproduce those of living amebæ, or to accept the statement that living protoplasm behaves exactly as any similar drop of fluid would under the same condition. He states that the currents set up in artificial amebæ by changes in surface tension are not the same as those in living ameba, contrary to Rhumbler and to Bütschli. The movement of ameba, he maintains, is not due to the flowing of the contents of the cell in a central, axial current out into the pseudopodium and back on the sides, as occurs in the artificial ameba; but rather to a rolling forward of the upper surface over the anterior edge to the lower surface, where it becomes fixed to the surface on which the ameba is crawling. The part played by surface tension, he claims, is in the case of amebæ a very subordinate one, and it is not sufficient to explain the movements of the living cell.

However the discussion concerning the amebæ may turn, it must be appreciated that there are some important differences between even the ameba and the leucocyte. The latter has by far the simpler organization, and approaches in structure, and presumably, therefore, also in response to stimuli, more closely to the simple drop of colloid matter. It has no pulsating vacuoles, no specialized pseudopodia, never forms shells or coverings, and does not conjugate as do the amebæ. The external surface of the leucocyte is much simpler, an important fact in connection with surface tension effects, for in the leucocyte the surface seems to be practically undifferentiated, naked protoplasm; whereas in ameba it is formed of a well-differentiated "ectosarc," which has marked motile powers, being able to contract sufficiently to cut an injured ameba completely in two. At the very least *the surface tension explanation of leucocytic action agrees perfectly with most of the observed actions of leucocytes*, and it is the only reasonable theory offered. There seems to be no middle ground between such a physical theory and a

¹ Publication No. 16, Carnegie Institute, Washington, 1904; also see *American Naturalist*, 1904 (38), 625.

metaphysical theory which would endow a single cell, without organs or nervous system, with the reasoning powers of highly developed animals, a position incompatible with the entire evidence of experience.

SUPPURATION

For the formation of pus two conditions are necessary : (1) the accumulation of leucocytes, and (2) necrosis and liquefaction of cells and tissue elements. Many leucocytes may be present in a tissue without suppuration ; *e. g.*, erysipelas. Necrosis of cells with their gradual liquefaction and absorption may also occur without suppuration ; *e. g.*, infarcts, aseptic liquefaction, necrosis, etc. Hence for suppuration to occur there must be produced substances with positive chemotaxis, to cause accumulation of leucocytes, for if a necrotic area is devoid of leucocytes, it does not suppurate ; *e. g.*, caseous tubercles. Secondly, necrosis must occur, for digestion and liquefaction of *living* cells and tissues does not take place. Only substance meeting these requirements—*i. e.*, causing positive chemotaxis and cell necrosis—will cause suppuration. Therefore, although bacterial infection is the usual cause of suppuration,¹ it may be produced by many other substances, among which the following are best known : Bacterial proteins, even from non-pathogenic bacteria ; oil of turpentine, mercury, croton oil, silver nitrate solutions (5 to 10 per cent.), and certain vegetable proteids (vegetable “caseins”).

An excellent example of the importance of leucocytes for suppurative softening is the caseous tubercle, which is usually free from leucocytes and does not undergo suppuration. If for any cause leucocytes are attracted into the caseous area, softening and pus formation promptly occur. Hence Heile² found that while pus from a “cold” tuberculous abscess will not digest fibrin and does not give the biuret reaction, both reactions appear after a leucocytosis has been brought about by injection of iodoform. It was formerly considered that the softening was due to the digestive action of the enzymes of the infecting bacteria, many of which were known to produce digestive enzymes dissolving proteid culture-media ; *e. g.*, *Staphylococcus pyogenes*. Although to some extent these enzymes may be a factor in causing the

¹ Buchner considers that bacteria will not produce suppuration unless they are broken down so that their *pyogenic proteids* are released ; *e. g.*, anthrax bacilli cause suppuration when acting locally, as in malignant pustule, but not when they are causing septicemia, because only in the former case are their pyogenic proteids liberated.

² Zeit. klin. Med., 1904 (55), 508.

softening of the fixed tissues and of the killed leucocytes, their effect is probably insignificant as compared with the enzymes liberated by the leucocytes, as shown by the production of active experimental suppuration under aseptic conditions with turpentine, croton oil, etc.¹ Suppuration is, therefore, the result of three processes: (1) Necrosis of cells; (2) local accumulation of leucocytes; (3) digestion of the necrotic cells, fibrin, and tissue elements by enzymes which are derived from three sources, as follows: (a) the leucocytes; (b) the infecting bacteria (if such are present); (c) the fixed tissue-cells. Possibly small quantities of enzymes are also introduced in the blood plasma, but these are probably very inconsiderable. Normal serum, and probably also normal cells, contain antibodies for the proteolytic enzymes of the leucocytes, and hence neutralization or destruction of these antibodies may be an important factor in determining the rate and amount of suppuration.²

The proteolytic enzymes of the leucocytes and tissue-cells have been previously considered in connection with the subject of autolysis (Chap. iii), and it is necessary here only to call attention to the fact that these enzymes are of at least two varieties: (1) Proteolytic enzymes of the polymorphonuclear leucocytes, which act best in alkaline medium (Opie³); (2) autolytic enzymes of the tissue-cells, which act best in an acid medium (Hedin, *et al.*). Possibly the mononuclear leucocytes contain, like the tissue-cells, enzymes acting in an acid medium. The antienzymatic action of pus serum is favored by an alkaline reaction, but is altogether lost in an acid medium (Opie).

COMPOSITION OF PUS

Because of its method of production, pus consists of the following substances: (1) The constituents of the exuded blood plasma; (2) the constituents of the leucocytes (and tissue-cells) that exist free in the pus; (3) the products of digestion of the proteids of the leucocytes and necrosed tissues. All analyses of pus that are recorded in the literature are in harmony with the above statements. In general the analyses consider pus as composed of two chief portions, the pus-corpuscles and the pus serum. As is to be expected, the composition of pus-corpuscles is simply that of a large mass of leucocytes, which contain minute quantities of substances taken up from the pus serum

¹ Apparently suppuration may occur in herpes zoster vesicles in the absence of bacteria, according to the findings of Kreibich (Wien. klin. Woch., 1901 (14) 583).

² See Opie, Jour. Exper. Med., 1905 (7), 316).

³ Jour. Exper. Med., 1906 (8), 410.

by absorption and phagocytosis. The old analyses of pus-corpuscles by Hoppe-Seyler¹ are given in the following table :

TABLE I.

Quantitative Composition of Pus-cells (in 1000 parts of the dried substance).

	I		II
Proteids	137.62	} 685.85	673.69
Nuclein	342.57		
Insoluble bodies	205.66		
Lecithin	..	} 143.83	75.64
Fat	..		75.00
Cholesterin	74.00		72.83
Cerebrin	51.99	} ..	102.84
Extractive bodies	44.33		

Mineral Substances in 1000 Parts of the Dried Substance.

NaCl	4.35
Ca ₃ (PO ₄) ₂	2.05
Mg ₃ (PO ₄) ₂	1.13
FePO ₄	1.06
PO ₄	9.16
Na	0.68
K	trace

As abnormal constituents of the leucocytes contained in abscesses may be mentioned glycogen, fat (from phagocytosis and from "fatty degeneration" of the leucocytes), and "peptone" (Hofmeister²).

Pus serum differs from blood-serum chiefly in the substances added to it through the proteolytic changes that occur in the pus. The fibrinogen that escapes from the vessels into suppurating areas becomes so altered that pus will not coagulate, even upon addition of fibrin ferment (defibrinated blood). The reaction of the serum is usually slightly alkaline, becoming strongly alkaline if much ammonia is produced, which occurs especially if there is secondary infection with the organisms of putrefaction. Sometimes, however, lipase derived from either bacteria or from the cells causes the splitting of sufficient amounts of fatty acids from the fats to make the reaction acid; lactic and other fatty acids are also sometimes formed. Presumably the nature of the infecting organism will modify the reaction, for some (*e. g.*, *staphylococcus*) cause an acid formation in media, while others (*e. g.*, *pyocyaneus*) causes an alkaline reaction. Hoppe-Seyler's analyses of pus serum gave the following results, which show no considerable deviation from the composition of blood plasma, except in an increased proportion of fatty matter and extractive substances.

¹ Med.-Chem. Untersuchungen.

² Zeit. physiol. Chem., 1880 (4), 268.

TABLE II.

	Quantitative composition of pus serum.		Plasma (normal).
	I	II	III
Water	913.7	905.65	908.4
Solids	86.3	94.35	91.6
Proteids	63.23	77.21	77.6
Lecithin	1.50	0.56	1.2
Fat	0.26	0.29	
Cholesterin	0.53	0.87	
Alcohol extractives	1.52	0.73	4.0
Water extractives	11.53	6.92	
Inorganic salts	7.73	7.77	8.1

Quantitatively the chief abnormal constituent of pus serum is the so-called "*pyin*" of the older writers, which is nucleoproteid derived from the decomposing leucocytes, and hence increasing in amount progressively with the age of the pus; it is characterized by its insolubility in acetic acid. The same substance is found more abundantly in the entire pus, on account of the presence of the cells, and when treated with 10 per cent. NaCl solution it forms a stringy mass which was formerly called "*Rovida's hyalin substance*." In the pus serum are found all the other constituents of the leucocytes, including particularly lecithin, cholesterin, fats (and soaps), cerebrin, "jecorin," and glycogen; and also the usual components of the blood-serum as well as some small quantities of pigment derived from decomposed red corpuscles.

The *products of autolysis* are of particular interest, and they are found in varying amount, but usually less abundantly than might be expected, probably because of their solubility and consequent rapid absorption. Albumoses and peptone seem to be constantly present (Shattock¹). The common occurrence of albumosuria during suppuration presumably depends on the absorption of digestion products from the pus,² but true peptone has not been satisfactorily identified in the urine. Leucin and tyrosin have also frequently been found in pus, but Taylor³ could find no workable traces of either monoamino- or polyaminoacids in a liter of pus, which may depend on their having been either absorbed or transformed into ammonium compounds. From the nucleoproteids *purin bodies* are formed and may be found in the pus. The relation of the purin bases to local leucocytosis is shown by Heile,⁴ who found in cold tuberculous abscesses

¹ Trans. London Path. Soc., 1892 (43), 225.

² Literature on albumosuria, see Yarrow, Amer. Med., 1903 (5), 452; Elmer, *ibid.*, 1906 (11), 169; Senator, International Clinics, 1905 (IV) series 14, p. 85. See also "Albumosuria," Chap. xix.

³ Univ. of California Publications (Pathol.), 1904 (1), 46.

⁴ *Loc. cit.*

a proportion of purin bases equal to 0.5 per cent., in similar abscesses after injection of iodoform, 1.57, and in acute supuration, 10.7. *Spermin* crystals are also occasionally found in old pus collections.¹ *Free fatty acids* and volatile fatty acids, such as butyric, lactic, valerianic, and formic, may also be present. Products of bacterial activity, such as bacterial proteids and pigments (*e.g.*, pyocyanin), may also be present. (See also discussion of "Autolysis of Exudates," Chap. iii).

All the numerous *enzymes* of the blood plasma, the leucocytes and the tissue-cells are present in pus. Thus Achalme² found evidence of the presence of the following enzymes in pus: proteolytic enzymes,³ lipase (splitting monobutyrim), diastase, rennin (coagulating milk), gelatinase, catalase, and oxidase, the last being very abundant. These seem to exist chiefly in the leucocytes, the pus serum being quite free from them. No evidence could be found of enzymes acting on amygdalin, saccharose, inulin, or lactose. Fibrin ferment is said to be absent from pus, which is quite surprising in view of the fact that this enzyme is generally considered as being derived chiefly from the leucocytes.

SPUTUM⁴

The chemistry of sputum may be properly considered in this connection. In reaction, sputum is ordinarily alkaline, but in case of marked bacterial decomposition in cavities the reaction may become acid. Its specific gravity varies from 1.008 to 1.026, usually varying directly with the number of leucocytes; the average specific gravity is about 1.013. The greenish color frequently observed depends generally upon blood-pigment (except in case of icterus), although in some instances the pigment is of bacterial origin. Renk⁵ has studied the proteids of sputum with special reference to the loss of proteid to the body and its relation to cachexia. In three patients (consumptives) studied, the daily amount of sputum of two averaged 145 grams for each; for the third it was 82 grams. This contained (average) 5 to 6 per cent. of solids; including mucin, 2-3 per cent.; proteid, 0.1-0.5 per cent.; fat, 0.3-0.5 per cent.; ash, 0.8-0.9 per cent. The daily loss of nitrogen was 0.75 gram,

¹ See Williams, Boston Med. and Surg. Jour., 1901 (145), 355.

² Compt. Rend. Soc. Biol., 1899 (51), 568.

³ Concerning proteolytic enzymes of pus see Opie, Jour. Exper. Med., 1906 (8), 410.

⁴ Complete bibliography given by Ott, "Chem. Pathol. der Tuberc.," Berlin, 1903.

⁵ Zeit. f. Biol., 1875 (11), 102.

which equals about 6 per cent. of the total daily nitrogen output of persons under condition of starvation.¹ Wanner² found characteristic variations in the amount of proteid in sputum from different conditions, as follows: in bronchitis the amount of proteid is very small; in bronchiectasis proteid is present, but the amount of uncoagulable nitrogen (due to autolysis) is relatively large; in phthisis as well as in bronchiectasis the amount of proteid does not exceed 1 per cent., in pneumonia it may reach 3 per cent., but it is highest in pulmonary gangrene. Any proteid content that causes more than a slight turbidity on boiling indicates an inflammation; *e. g.*, in case of doubt between a diagnosis of pneumonia and infarct a high proteid content speaks for the former. The mucin of sputum yields 33.6 per cent. of *glucosamin* when split with HCl, which gives an index of the quantity of mucin; this is highest in chronic bronchitis and lowest in pneumonia and phthisis. Kossel found 0.1–0.33 gm. of nucleins in the sputum daily.

The following table by Bokay (taken from Ott) gives the proportion of the organic constituents of sputum in parts per thousand:

TABLE III.

	Bronchitis in typhoid.	Fibroid phthisis.	Phthisis, early, in apex.	Phthisis, cavities.	Phthisis, advanced.	Phthisis, advanced.
Fatty acids as fat	0.224	0.845	0.462	2.468	3.468	9.725
Free fatty acids	trace	0.184	0.521	0.370	0.307	0.902
Soaps.	traces	0.380	0.430	0.537	0.516	3.973
Cholesterin	traces	0.4	1.617	0.172	1.160	0.141
Lecithin	traces	traces	1.543	. .	1.165	1.245
Nuclein	traces	0.102	. .	. .	0.260	0.489
Proteid	0.898	2.040	. .	4.430	3.455	5.115

On account of the digestion of the exudates by the leucocytes, sputum contains proteoses, peptones, and amino-acids, generally in proportion to the richness of the exudate in leucocytes; they are, therefore, most abundant in pneumonia. Simon³ found considerable albumose in phthisical sputum, but no nucleohiston or free histon. Staffregen, however, could find no true peptone in phthisical sputum, but Stadelmann⁴ found that such

¹ Plesch (Zeit. exp. Path. u. Ther., 1906, Bd. iii, July) found that 4.8 per cent. of all the absorbed calories were lost in the sputum in an advanced case of phthisis.

² Deut. Arch. klin. Med., 1903 (75), 347.

³ Arch. exp. Path. u. Pharm., 1903 (49), 449.

⁴ Zeit. klin. Med., 1889 (16), 128.

sputum contained enzymes hydrolyzing fibrin, and attributed this largely to bacteria.

The amount of *fats* seems to depend directly upon the number of pus-corpuscles and the age of the pus (*i. e.*, the amount of fatty degeneration). Jacobson found from 0.08 to 1.6 grams of fatty matter per day, containing on an average 14.76 per cent. of soaps, 15.79 per cent. of higher fatty acids, 0–10 per cent. of water-soluble fatty acids, 13.58 per cent. lecithin, and 10.49 per cent. cholesterin.

As to the *inorganic substances*, Bamberger found two types of sputum, catarrhal and inflammatory. In the inflammatory there is a deficiency in alkali phosphate, SO_3 constitutes more than 8 per cent. of the salts, and the ratio, $\frac{\text{Na}_2\text{O}}{\text{K}_2\text{O}}$ equals $\frac{15}{41}$. In catarrhal sputum the alkali phosphates constitute 10–14 per cent., $\frac{\text{Na}_2\text{O}}{\text{K}_2\text{O}} = \frac{31}{20}$, and the SO_3 is from 0.6–1.2 per cent. Chlorine is about the same in both forms. The results of his analyses are shown in the following table:

TABLE IV.

	Chronic phthisis.	Acute phthisis.
Water	94.55	93.38
Organic matter	4.67	6.88
Inorganic salts	0.78	0.74
One hundred parts of the salts contain:		
Chlorine	35.78	33.40
SO_3	0.70	0.80
P_2O_5	13.05	14.15
K_2O	24.07	19.99
Na_2O	27.90	31.69
Calcium phosphate	1.63	4.32 ¹
Iron phosphate	0.09	0.14
Magnesium phosphate	1.20	..
Ca and Mg carbonate and sulphate	1.74	0.22
Silicic acid	0.9	0.3

PROLIFERATION AND REGENERATION

The factors that incite cells to proliferate, as well as those that cause the cessation of proliferation after it has once started, are too entirely unknown to permit of speculation as to their exact nature. It seems probable, however, that they are, as

¹ Including magnesium.

Ziegler says, "identical with the stimuli which excite or increase functional and nutritive activity," and these are certainly in many instances of chemical nature. Thus the application of various irritating substances in not too concentrated a form (*e. g.*, painting the skin with iodine) may lead to proliferation without causing discernible degeneration of the cells. Mallory's¹ observations on the phenomena of proliferation and phagocytosis show that the same bacterial products which destroy the cells when concentrated, when sufficiently dilute cause proliferation of similar cells. Many other instances of proliferation in response to chemical stimuli might be cited, but in nearly all cases it is extremely difficult to determine that the proliferation is not, after all, reparative in compensation for degenerative changes, and, therefore, possibly obeying some other biological law than that of a simple reaction to a chemical stimulus.

Although proper nutrition is necessary for cell proliferation, yet it does not seem that excessive nourishment can lead to excessive cell multiplication, or by itself cause cell proliferation to take place. Oxygen and certain inorganic salts are essential for cell division even in the lowest forms, and among such simple organisms as sea-urchins and certain other marine forms segmentation of the unfertilized ova may be incited by changes in osmotic concentration, leading eventually to formation of perfect larvæ (J. Loeb, *et al.*²). Potassium salts seem to be particularly important for proliferating cells, and Beebe and also Clowes and Frisbie³ have found that actively growing malignant tumors are rich in potassium and poor in calcium, whereas in slow-growing tumors the reverse is the case. Dennstedt and Rumpf⁴ also found that in hypertrophy of the heart the amount of potassium is increased, while in chronic degeneration of the myocardium the calcium and magnesium are usually increased.

Chemical studies of proliferation are lacking, except in regard to the development of the embryo, etc. New tissues differ from adult tissues in having a large proportion of water, and in having a larger proportion of the "primary" cell constituents and a smaller proportion of the various secondary constituents, since these last are largely products of the activity of the adult cell. Of the primary constituents, the proportion of the nucleoproteids is particularly high, and a number of interesting facts

¹ Jour. Exp. Med., 1900 (5), 15.

² See J. Loeb, Studies in General Physiology, Chicago, 1905.

³ See "Tumors," Chap. xvii.

⁴ Zeit. klin. Med., 1905 (58), 84.

concerning the nucleoproteids in cell division have been determined. Most important, perhaps, are the classical observations of Miescher, who found that during the migration of salmon up stream to the spawning grounds, during which time no food is taken, the proteids of the muscular tissue become largely transformed into the protamin type of proteid (characterized by containing large proportions of the polyamino-acids, such as arginin, histidin, and lysin¹), which unite with nucleic acids to form the abundant nucleoproteid of the spermatozoa and ova. Whether such a transformation of proteids occurs in mammalian cells during cell multiplication cannot be stated, but certainly from some source an additional supply of nucleoproteid is derived. The nucleoproteids during karyokinesis undergo a chemical change in that they become of a more acid type (presumably through splitting off of part of the proteids from the nucleic acid), which results in the characteristic increase in affinity for basic dyes. This suggests the participation of an enzyme in the process of karyokinesis, just as there seems to be in the production of pycnosis in degenerating cells, but there seems to be no conclusive evidence on this point. Gies² could find no enzyme in spermatozoa that incites cell division in the ova of sea-urchins (*Arbacia*).

In *metaplasia* we have what may be interpreted as a chemical alteration due to mechanical stimuli, *e. g.*, the formation of keratin by cells that ordinarily do not do so; the deposition of calcium salts and osteoid transformation of connective tissue in rider's bone, etc. That such is the case, however, cannot be positively stated from the evidence at hand.

¹ Concerning protamins, see résumé by Kossel, *Biochem. Centr.*, 1906 (5), 1 and 33.

² *Amer. Jour. Physiol.*, 1901 (6), 54.

CHAPTER XI

DISTURBANCES OF CIRCULATION AND DISEASES OF THE BLOOD

THE COMPOSITION OF THE BLOOD

THE function of the blood being to maintain an equilibrium in the temperature, chemical composition and osmotic pressure between all parts of the body, it follows that it is never of exactly the same composition in any two places or at any two times. To the extent that every tissue is continually giving off something to the blood, we may consider that every organ is a factor in its formation, and as a result of this multiplex origin of the blood, the substances it may contain are beyond enumeration. There are probably but few chemical substances occurring in the tissue-cells that do not also occur in greater or less amount in the blood. In addition to these there are also the substances characteristic of the blood itself, besides a host of substances of unknown nature, apparently manufactured in response to the stimulation of substances entering the body from outside; for we find that the blood of every adult individual contains substances that make him immune to a multitude of diseases that he has had in childhood, as well as substances that in later life protect him to a greater or less degree from infection by such organisms as the colon bacilli of his intestine, the pneumococci and streptococci in his throat, etc. We have learned of these defensive substances within very recent times, and also of the "antienzymes" that possibly protect the blood from the digestive enzymes of the body cells. What other substances of importance we may yet find in the blood is an open question. There are no apparent limits to the possibilities of the study of the blood, for it represents a little of every organ, and a good deal that is characteristic of itself.

In discussing briefly the substances that have been isolated from the normal blood, before considering the changes that occur in it during pathological conditions, we may roughly divide the blood into the formed elements and the plasma in which they are suspended.

The Formed Elements.—By weight, the red corpuscles constitute from 40 to 50 per cent. of the blood, the percentage varying under different conditions, while the total weight of the leucocytes and platelets is insignificant. The hemoglobin constitutes from 86 to 94 per cent. by weight of the solids of the red corpuscles, but the physical and chemical relations that it bears to the stroma of the corpuscles are as yet undetermined (see "Hemolysis"). Of the remaining constituents of the corpuscles, from 5 to 12 per cent. consist of proteids, probably chiefly *globulins* and *nucleoproteids*; 0.3 to 0.7 per cent. of *lecithin*; and about 0.2 to 0.3 per cent. of *cholesterin* (Hoppe-Seyler). The outer coat of the red corpuscles does not seem to be equally permeable for all substances, and therefore we find the composition of the fluid portion of the cell quite different from that of the plasma about it. The *salts* of the corpuscles consist largely of potassium phosphate, a little sodium chloride, some magnesium, but no calcium, which is quite different from their proportion in the plasma. Probably many of the other constituents of the plasma, especially urea, penetrate the red corpuscles to a greater or less degree, but most of them, particularly the sugar, remain chiefly in the plasma.

Hemoglobin, the most characteristic constituent of all the heterogeneous components of the blood, is a compound proteid, and probably exists combined with some other constituent of the corpuscle, most probably the lecithin. It splits up readily into a proteid, *globin*, and an iron-containing substance, *hemochromogen*, which readily takes up oxygen to form *hematin*. Only about 4 to 5 per cent. of the hemoglobin is hemochromogen, and iron constitutes but about 0.4 per cent. Hematin may be further split up into other substances, which will be considered in the discussion of "Hemorrhage."

The **leucocytes** consist chiefly of nucleoproteids, with probably some globulin, and they also contain glycogen, lecithin, and cholesterin. The *blood-platelets* are believed to be largely nucleoproteid, but little is known of their actual composition.

Blood plasma differs from blood-serum in that the latter is formed from the former through the conversion of the fibrinogen into fibrin. Serum, therefore, contains no fibrinogen, but more fibrin ferment; otherwise it is practically the same as the plasma.

Proteids.—*Fibrinogen* has the general properties of a globulin, with also a peculiar tendency to go into the insoluble form, *fibrin*. (This process will be discussed under "*Thrombosis*.") In the plasma are also other globulins, one soluble in water (*pseudo-globulin*), the other insoluble in water (*euglobulin*). *Serum-albumin*, another proteid of the plasma, probably consists of two or more varieties of albumin. There are also nucleoproteids (*prothrombin*) and non-coagulable proteids, which being poorly understood have been variously considered as glycoproteids, or mucoids, or albumoses.

Other Constituents.—The *fat* of the plasma varies much according to the time which has elapsed after the taking of food; in fasting animals it amounts to from 0.1 to 0.7 per cent. The sugar fluctuates less, being normally about 0.1 per cent., while the urea has been estimated at 0.05 per cent. Most of the sugar is dextrose; but probably there is some levulose, possibly some pentose and other forms, and possibly also sugar combined with lecithin (*jecorin*) or other substances. Soaps, cholesterin, and lecithin also exist free in the plasma.

Plasma differs strikingly from the corpuscles in that its inorganic substances are chiefly sodium and chlorine, while potassium and phos-

phoric acid are almost entirely absent. Another important fact is that when the plasma is combusted, the acid radicals remaining do not suffice to balance the bases, indicating that much of the inorganic bases is joined with organic substances, probably as ion-proteid compounds. The alkali joined to the proteid is non-diffusible, and constitutes about five-sixths of the total alkali.

The **concentration of the electrolytes** of the blood has been determined by ascertaining the lowering of the freezing-point, which in human blood averages about 0.526° ; this corresponds closely to the effect of a salt solution of 0.9 per cent. strength. About three-fourths of the dissolved molecules of the blood-serum are electrolytes, and about three-fourths of these are molecules of NaCl, most of which are in the dissociated state.¹

Enzymes.—A large number of enzymes exist in the blood, the following having been detected: *diastase, glucase, lipase, thrombin, rennin, and proteases*. The proteases are held in check to a large extent by “*antiferments*” that are also present (see “Enzymes,” p. 72). In relation to the antiferments are the innumerable antibodies that exist normally in the serum for foreign proteids, foreign cells, and for bacteria and their toxins, as well as those resulting from reaction to infection, etc.

The proportions in which the constituents of the plasma normally occur have been determined by Hoppe-Seyler and by Hammarsten as follows:²

TABLE I.

	No. 1.	No. 2.
Water	908.4	917.6
Solids	91.6	82.4
Total proteids	77.6	69.5
Fibrin	10.1	6.5
Globulin	..	38.4
Seralbumin	..	24.6
Fat	1.2	12.9
Extractive substances	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

No. 1 is an analysis by Hoppe-Seyler.

No. 2 is the average of three analyses made by Hammarsten.

Alkalescence.—It is very difficult to determine the exact alkalinity of the blood plasma. If we titrate with an acid, we liberate much of the alkali from the proteids, dissociate all the Na_2CO_3 present, as well as the NaHCO_3 and the sodium phosphate, and find in this way that the entire fresh blood contains *neutralizable alkali* corresponding to a solution of Na_2CO_3 of about 0.443 per cent. strength (Strauss). In other words, the blood has a quantity of alkali in combination that can be drawn

¹ Concerning relation of conductivity to freezing-point see Wilson, Amer. Jour. of Physiol., 1906 (16), 438.

Concerning the viscosity of the blood see Burton-Opitz, Pflüger's Arch., 1906 (112), 189; and Determann, Zeit. klin. Med., 1906 (59), H. 2-4.

² For complete analyses of the blood see Abderhalden, Zeit. physiol. Chem., 1898 (25), 106.

upon to neutralize acids to the extent indicated by the above figures. The *real alkalinity* of a fluid, however, is dependent upon the number of free OH ions in the solution; and Höber has determined by physico-chemical methods that the concentration of OH ions in blood is but little greater than in distilled water.¹

The alkali of the blood exists in part as alkaline salts, carbonate and phosphate (the *diffusible alkali*), and partly combined with proteid (*non-diffusible alkali*). As the corpuscles are richer in diffusible alkali than the plasma or serum, the number of corpuscles modifies the alkalinity of the blood decidedly. Much importance is attached to the question of the alkalinity of the blood for two reasons:² first, in certain conditions of disease the blood contains so much of organic acids that the alkali is partly saturated and the power of the blood to carry CO₂ is lessened, with serious results (see "Acid Intoxication," Chap. xviii); and, second, the bactericidal power of the blood is found to vary according to its alkalinity.³ In fact, metabolic activity seems generally to be favored by certain degrees of alkalinity; for example, J. Loeb⁴ found that sea-urchin eggs develop with much greater rapidity if a small amount of OH ions is free in the sea-water. It is stated that in febrile conditions the alkalinity of the blood is reduced,⁵ but the methods available for determining blood alkalinity are too unreliable to decide this point satisfactorily.⁶ Brandenburg⁷ states that the nondiffusible alkali varies according to the amount of proteid in the blood; in pneumonia and acute nephritis he found it low. Rzentkowski⁸ also attributes the reduced power of the blood to neutralize acids, which he observed in acute infectious diseases and in uremia, to decreased quantity and altered quality of the blood proteids. Libman⁹ has suggested that the acids produced by the bacteria themselves may be a factor in reducing the alkalinity of the blood in infection. Orlowsky¹⁰ could find no

¹ Pflüger's Arch., 1900 (81), 535.

² For bibliography on Alkalinity of Blood see v. Limbeck, "Klinische Pathol. des Blutes," 1896; and Hamburger, "Osmotischer Druck und Ionenlehre," 1902.

³ See Hamburger, *loc. cit.*, p. 280.

⁴ Arch. f. Entwicklungsmechanik, 1898 (7), 631.

⁵ See v. Limbeck, *loc. cit.*; see also Orlowsky, Deut. med. Woch., 1903 (29), 601.

⁶ Kireeff (Cent. f. inn. Med., 1905 (26), 473) claims that in most febrile conditions the alkalinity as determined by titration is normal or slightly lowered, except in typhus (Flecktyphus), in which he finds it always increased.

⁷ Deut. med. Woch., 1902 (28), 78; Zeit. f. klin. Med., 1902 (45), 157.

⁸ Arch. exp. Path. u. Pharm., 1906 (55), 47.

⁹ Jour. Med. Research, 1901 (6), 84.

¹⁰ *Loc. cit.*

decrease in the alkalinity of the blood in leucocytosis, or when virulent bacteria were introduced into the blood. Awerbach¹ claims that in severe high fevers the bactericidal effect of the blood alkalinity is increased (see also "Passive Congestion" for further discussion concerning the relation of alkalinity to bactericidal power).

HEMORRHAGE

Hemorrhages result from an altered condition in the vessel-walls, which may be due either to trauma or to chemical injuries. Of the chemical agencies causing hemorrhages, bacterial products are the most important practically, but many poisons, such as phosphorus, formalin, *phytotoxins* (ricin, abrin, and crotin), and *zoötoxins* (snake venoms) cause numerous and abundant hemorrhages. Formerly, the tendency was to ascribe hemorrhages from the above causes to mechanical injury of the vessels by thrombi, or by emboli of agglutinated corpuscles, but the work of Flexner² has shown that venoms cause hemorrhages by injuring the capillary walls, so that actual rents are produced by the intravascular pressure, and it seems highly probable that hemorrhages are produced by other chemical substances in a similar way. We may, therefore, refer such hemorrhages to an *endotheliotoxic* action of the poison, or to a solvent effect upon the intercellular cement substance. In the case of ordinary chemical poisons the endotheliotoxic action is not specific, but with some of the toxins it seems to be quite so; for example, rattlesnake venom contains an endotheliotoxic substance (*hemorrhagin*), which seems to be a specific poison for endothelium, and which is the most dangerous constituent of the venom. If we immunize animals against tissues containing much endothelium (*e. g.*, lymph-glands), their serum will be found to contain endotheliotoxins, so that when this serum is injected subcutaneously into a susceptible animal, large local hemorrhages result; if injected into the peritoneal cavity, there results marked desquamation of the endothelial cells, which soon undergo degenerative changes (Ricketts³). It is quite probable that the bacterial poisons that cause marked hemorrhagic manifestations likewise contain endotheliotoxins, although this matter does not seem to have been investigated.

Even *hemorrhage by diapedesis* seems to be due to, or at least associated with, chemical changes in the capillary walls, for

¹ Med. Obosrenije, 1903, p. 596.

² Univ. of Penn. Med. Bull., 1902 (15), 355.

³ Trans. Chicago Path. Soc., 1902 (5), 181.

Arnold¹ found that when capillaries from which diapedesis had occurred were stained by silver nitrate, dark areas were found between the endothelial cells. As silver nitrate is a stain for chlorides, and darkens intercellular substance because it is rich in sodium chloride (Macallum), it is probable that there is an increase in the amount or a difference in the method of combination of the chlorides of the cement substance between the endothelial cells, at the places where red corpuscles escape.

Hemorrhage in cachectic conditions is often ascribed to changes in the vessel-walls due to malnutrition, but it is difficult to imagine capillary walls suffering from lack of nourishment, even with the poorest of blood, and it seems more probable that the hemorrhages are due, even in cachexia, to chemical constituents of the blood that injure the endothelium.

Changes in the Extravasated Blood.—These begin soon after its escape. In most situations sufficient fibrin ferment is formed to lead to prompt clotting, but in the pleura and other serous cavities the blood may remain fluid for some time, probably because of lack of cellular injury that might cause liberation of fibrin ferment. If the blood does not become infected, the rapidity of subsequent changes depends chiefly upon the location and amount of blood. Small extravasations of blood into the tissues are subjected to the action of the tissue cells and of leucocytes emigrating freely from the capillaries; large masses of blood are but little affected by these agencies, the leucocytes within the mass soon die, and secondary changes go on very slowly. In small subcutaneous hemorrhages (*e. g.*, a bruise) enzymes from the invading leucocytes and tissue-cells soon dissolve the small quantities of fibrin present; even earlier the stroma of the red corpuscles is so altered that hemolysis occurs and the hemoglobin escapes and diffuses into the tissues. This hemolysis may be brought about by the action of proteolytic enzymes on the corpuscles, or by the hemolytic action of the products of proteid splitting. Soon the hemoglobin disintegrates, forming the masses of pigment so characteristic of old hemorrhagic areas, and also giving rise to the discoloration observed beneath the skin in the later stages of resorption of hemorrhagic extravasations. The first products of the splitting of hemoglobin are: (1) The proteid, *globin*, which constitutes 94 per cent. of the hemoglobin; and (2) the iron-containing coloring-matter, *hematin* (in the absence of oxygen the pigment is *reduced hematin* or *hemochromogen*). As hematin may be experimentally obtained by the action of proteases upon hemo-

¹ Virchow's Arch., 1875 (62), 157.

globin, the decomposition of the hemoglobin in the tissues is probably accomplished in a similar way by the proteases of the leucocytes, tissue-cells and blood plasma; the globin is thus digested away and the soluble products carried off, while the insoluble hematin remains.¹ The hematin gradually undergoes further changes, forming an iron-free pigment (*hematoidin*) and an iron-containing pigment (*hemosiderin*).

Hematoidin is nearly or quite identical with the bile-pigment, *bilirubin*, and is absorbed from the hemorrhagic extravasation and eliminated as bilirubin in the bile. Possibly some of the hematoidin undergoes transformation into *urobilin*, and is then eliminated in the urine. *Hemosiderin* seems to be relatively insoluble and, therefore, is more slowly removed, so that it may be found at the site of a hemorrhage after the other evidences of blood extravasation have been removed. It may be easily demonstrated by staining with potassium ferrocyanide, the Prussian blue that is formed being readily distinguished. Unstained hemosiderin generally appears in the form of brown or yellowish-brown granules, never as crystals. After a time the hemosiderin is taken away, and probably is to a greater or less extent deposited in the liver and spleen, either as hemosiderin or as some other iron compound. Eventually it is probably utilized to make new hemoglobin; at any rate, the iron liberated by the breaking up of hematin within the body does not appear to be eliminated.²

The changes in the red corpuscles described above are not at all peculiar to extravasated blood, but are quite the same as the changes that are going on continuously and normally in the blood. Red corpuscles are short-lived, being but non-nucleated fragments of cells, and they are continually disintegrating with the production of iron-free pigments that are excreted as the coloring-matters of the bile and the urine, while the iron is worked over again into new hemoglobin after a varying period of storage in the tissues, particularly in the spleen and liver. The destruction of red corpuscles under normal conditions seems to take place chiefly in the spleen, bone-marrow, and hemolymph glands, where injured or decrepit corpuscles are taken out of the blood by the phagocytic endothelial cells, and decomposed by intracellular enzymes. In hemorrhagic extravasations the changes are essentially the same; some corpuscles are destroyed by phagocytes, but more by extracellular enzymes. The products of decomposition also seem to be no different from those

¹ More fully discussed in the consideration of "Pigmentation," Chap. xvi.

² See Morishima, Arch. f. exp. Path., 1898 (41), 291.

formed during normal katabolism of hemoglobin, and they meet the same fate in the end.

If the hemorrhages are very abundant, some hemoglobin may be absorbed as such and appear in the urine, but this probably seldom happens unless red corpuscles are also being destroyed in the circulating blood. An increased amount of iron accumulates in the liver, but if much blood has been lost by hemorrhage on free surfaces, the iron content of the liver is decreased, as it is taken away to form new hemoglobin (Quinke¹). Excretion of bile-pigments is increased by destruction of blood (Stadelmann), but not greatly in the case of hemorrhages, for the blood is decomposed and absorbed too slowly. Schurig² found that hemoglobin injected into the tissues is partly decomposed *in situ* with formation of iron compounds, but the greater part enters the circulation as hemoglobin, and is partly converted into bile-pigment by the liver-cells, the rest being converted into iron compounds by the spleen, bone-marrow, and renal cortex.

If the hemorrhagic extravasation has been large in amount, the deeper portions of the mass are not soon, if ever, invaded by leucocytes or tissue-cells. Consequently the blood is acted upon very slowly by the enzymes liberated by the leucocytes it contains itself, and by the small amounts of proteases in the serum. Furthermore, the products of decomposition are not soon absorbed, but accumulate in considerable amounts, so that we often find crystalline deposits of hematin, sometimes even of hematin, hemoglobin, or *parahemoglobin* (Nencki³) or *methemoglobin*.

The least soluble constituent of the red corpuscle stroma, *cholesterin*, also accumulates in such extravasations as large, thin plates; after most of the other products of disintegration have been absorbed from such accumulations of blood, the most conspicuous part of the residue may be a mass of cholesterin crystals imbedded in proliferating connective tissue.

HEMOPHILIA⁴

Since hemophilia seems, superficially at least, to depend upon some alteration in a chemical property of the blood, namely, coagulability, it is frequently regarded as an example of hereditary transmission of a chemical peculiarity. The exact cause

¹ Deut. Arch. klin. Med., 1880 (25), 567; 1880 (27), 193.

² Arch. exp. Path. u. Pharm., 1898 (41), 29.

³ Arch. exp. Path. u. Pharm., 1886 (20), 332.

⁴ Literature and résumé given by Stempel, Cent. f. Grenzgeb. Med. u. Chir., 1900 (3), 753; Sahli, Zeit. f. klin. Med., 1905 (56), 294.

of this peculiar tendency to prolonged bleeding from insignificant or perhaps imperceptible wounds has been sought vigorously by both histological and chemical means, but as yet without avail. Various observers have described abnormal thinness, or increased cellularity or fatty degeneration of the vessel-walls, but the findings have been far too inconstant to afford a satisfactory anatomical explanation of all the features of hemophilia. Likewise increased blood pressure can be ruled out, for although the left heart is frequently enlarged, there is usually no increased blood pressure demonstrable; furthermore, conditions of high blood pressure, such as nephritis, do not cause hemophilia. The theory of "hydremic plethora" is also without good foundation.

The most natural place to look for the fundamental fault is in the blood, but speaking strongly against this is the frequent occurrence of "local" hemophilia; *e. g.*, in this type of hemophilia wounds of the skin may behave as in normal individuals, whereas any injury of the mucous surfaces is followed by pronounced hemophilic bleeding;¹ in other cases the hemophilic bleeding is limited to regions above the shoulders; in still another class the bleeding is always from one organ, *e. g.*, the kidneys. Nevertheless, a great deal of investigation of the blood has been done, chiefly with negative results. There are no characteristic changes in the cellular elements of the blood, beyond the changes common to all secondary anemias, excepting possibly a decrease in the number of white corpuscles with a relative increase in the number of lymphocytes as observed by Sahli. No constant alterations in the salts of the blood have been found; and the proportion of water, the alkalinity, and the osmotic pressure of the serum all seem to be normal. Since bleeding is normally stopped principally by coagulation, a deficiency in fibrin or its antecedents might be expected, but most studies on this point have shown a normal amount of fibrinogen in the blood of hemophiliacs, the frequent formation of large tumors of clotted blood at the bleeding points supporting the experimental evidence that the blood contains an abundance of fibrinogen. As to the rate of clotting, the results obtained by different observers are by no means in accord, which seems to be explained by the recent studies of Sahli,² who has avoided a number of errors made in earlier investigations. He found that in the intervals between the attacks of hemorrhage the rate of the coagulation of the blood is constantly much slower than normal. During an attack of bleeding the coagulation time approaches the normal; indeed, it may be faster than normal;

¹ Abderhalden, Ziegler's Beitr., 1904 (35), 213.

² *Loc. cit.*

apparently this is due to a reaction on the part of the organism to the loss of blood. If blood is collected directly from the site of bleeding the coagulation time is very rapid, because of the accumulation of fibrin ferment from the clot over which the escaping blood flows. Yet in spite of the normal coagulability of the blood and the rapid clotting after the blood escapes from the vessel, bleeding continues for long periods before it can be stopped. As there is no general change in the properties of the blood to account for the bleeding, and as local influences seem to be important in hemophilia, Sahli advances the plausible hypothesis that *chemical changes in the vessels* must be the essential factor in hemophilia. Hemorrhage is ordinarily checked chiefly by the formation of clots that plug up the bleeding vessels at the point of the hemorrhage. The local formation of a clot is believed to be due to liberation of fibrin-ferment (or its antecedents) by the injured cells of the vessel-wall at the point of the vascular lesion. If the cells of the vessel-wall are deficient in these fibrin-forming substances, the blood will not clot in the mouths of the vessels, but will first clot when it reaches a place where fibrin-forming substances are furnished by other tissues, or, as is generally the case, when the leucocytes are broken up by exposure to the air or other injurious influences so that they liberate fibrin-ferment. Under these conditions the blood may clot in large masses, but as there is no fibrin adhering to the vessel-walls at the bleeding openings, blood continues to escape. Sahli considers, therefore, that the cause of hemophilia lies in hereditary deficiency of the fibrin-forming substances, thrombokinasase or zymoplastic substance (see "Thrombosis"), in the vessel-walls, so that when the vessels are injured there is no local production of fibrin such as occurs normally. Local hemophilia may be explained readily as a local deficiency in fibrinoplastic material. In general hemophilia even the leucocytes may exhibit the same defect, in which case clotting of the blood is diminished even outside the tissues. This hypothesis seems to be in excellent agreement with the facts now known, but there yet remains to be demonstrated such a lack of fibrin-forming elements in the vessel-walls and other tissues of a hemophilic subject. This hypothesis perhaps also explains why the marked increase in coagulability of the blood obtained by administration of calcium salts (Wright¹) is, as Wright's observations show, not sufficient alone to stop hemophilic bleeding, even though the rapidity of clotting is much greater than normal.

¹ Brit. Med. Jour., 1894 (ii), 57.

ANEMIA AND THE SPECIFIC ANEMIAS

The customary division of the anemias is into—(a) *primary*, i. e., those in which the cause seems to depend upon some abnormality in the blood-forming organs or in the blood itself; and (b) *secondary*, embracing anemias the result of some obvious cause, such as hemorrhage, poisoning with blood-destroying poisons, cachexia, etc. In these various forms of anemia certain chemical differences prevail, but they are by no means so striking as are the histological differences in the formed elements of the blood.¹

SECONDARY ANEMIAS

As the simplest variety, anemia following a single large hemorrhage may be considered first.

If loss of blood by hemorrhage is rapid, the effects are naturally much more serious than when the loss is slow. The total quantity of blood in the average adult is estimated at about $\frac{1}{13}$ to $\frac{1}{15}$ the total body weight (therefore about 10 to 12 pounds), although this proportion does not hold for extremely obese or extremely thin individuals;² in infants the proportion is lower—about $\frac{1}{20}$. When one-third of the total amount of blood is lost rapidly, a marked fall of blood pressure occurs; loss of one-half of the total amount may be fatal, and loss of more than that at one time usually is fatal. The chief cause of death following large hemorrhages is the low blood pressure rather than the loss of any of the constituents of the blood; hence the successful results of the use of physiological salt solution after severe hemorrhage. The number of corpuscles may be greatly reduced after several small hemorrhages, even to as low as 11 per cent. of the normal number (Hayem), without fatal results, because in the intervals between the hemorrhages enough fluid has been taken up by the blood to maintain the blood pressure within safe limits.

After a severe hemorrhage the composition of the blood changes rapidly, for the fluids contained within the tissues and lymph-spaces pass into the blood in large amounts. This helps to maintain blood pressure, but results in the blood containing a larger proportion of water and salts and a smaller amount of proteid and red corpuscles; the "total alkalinity" also falls, largely because of the scarcity of "fixed alkali," on account of the poverty in corpuscles and blood proteids. The proportion of water increases at first more rapidly than the proportion of

¹ Concerning local anemia, see "Infarcts."

² Haldane and Smith (Jour. of Physiol., 1900 (25), 331) estimate the blood of adults at but $\frac{1}{20}$ of the body weight.

salts, and as a consequence the size of the red corpuscles is increased because of imbibition of water; indeed, it is possible that this may even be sufficient to cause hemolysis, which will happen if the isotonic strength of the blood becomes less than that of a 0.46 per cent. NaCl solution (Limbeck), while swelling may occur whenever the strength is below 0.8 per cent.

Regeneration of the blood begins very soon, and for some time the number of corpuscles exceeds the proportion of hemoglobin. During this time the amount of iron in the liver and spleen is decreased, it being taken up to be used in the formation of new hemoglobin. If the hemorrhages are numerous and the condition of anemia prolonged, secondary changes in the viscera may occur, fatty metamorphosis being most marked, supposedly because of decreased oxidation. Indeed, many observers state that repeated bleedings greatly increase body weight by causing increased fat deposition.

Metabolic Changes.—Gies¹ studied the *metabolism* of dogs after withdrawing a total amount of blood equal to 11.5 per cent. of the body weight during four bleedings, and found that a slight and temporary increase in nitrogenous elimination followed the bleedings, owing to an increased proteid katabolism. Sugar increases in the blood, while albumin and lactic acid appear in the urine. After each successive hemorrhage the proportion of fibrin and the coagulability of the blood increase, while the proportion of the ash obtained from both blood and serum remains practically unchanged (Meyer and Gies). Baumann² states that in regeneration after hemorrhage the serum albumins increase more rapidly than the globulins, while others have observed the opposite relation. The urine in secondary anemia shows the effects of increased proteid katabolism, its specific gravity, total solids, and total nitrogen being raised; the total amount of urine is at first diminished because of lowered blood pressure, but it soon rises above normal and later falls back to normal. The view formerly held that oxidation is decreased in anemia has been considerably modified by more recent investigations.³

Secondary anemia due to cachexia, or to malnutrition, is accompanied by a general decrease in all the elements of the blood, both cellular and chemical. The proteids of the plasma, particularly, show a decrease in starvation, being drawn on by the cells for food, and the total quantity of blood as well as of

¹ American Med., 1904 (8), 155 (résumé of literature).

² Jour. of Physiol., 1903 (29), 18.

³ See Mohr, Zeit. exp. Path., 1906 (2), 435.

each of its constituents is decreased (Panum¹), but the proportion of blood to body weight remains about normal.

Anemia due to hemolytic agencies presents quite different features, in that the red corpuscles are almost solely attacked and the products of their disintegration are present in the plasma. As a result, the plasma or serum may contain free hemoglobin, and if the hemoglobin is in large amounts, it may escape into the urine. Thus *paroxysmal hemoglobinuria* is probably due to the presence in the blood of hemolytic substances, which can be demonstrated in the blood of the patients during the attack.² The products of the decomposition of the hemoglobin set free by hemolysis are present not only in the blood, but also in the organs, particularly the liver and spleen, which become rich in iron. Excretion of bile-pigments also increases, and "*hematogenous jaundice*" may result, the bile-pigments that are present in the blood being derived from the hematin of the hemoglobin molecule. Changes in metabolism occur which are quite similar to those observed in other forms of anemia, with fatty changes in all the parenchymatous organs, increased proteid katabolism, and an excessive quantity of pigmentary substances, particularly urobilin, in the urine.

CHLOROSIS

The characteristic feature of the blood in chlorosis is the relatively small amount of hemoglobin in proportion to the number of corpuscles. Apparently, therefore, the fault lies rather in the manufacture of hemoglobin than in either a destruction or a deficient formation of red corpuscles. Erben's³ analyses of chlorotic blood showed that the total amount of proteid is decreased, chiefly because of the deficiency of hemoglobin; the relation of serum globulins and serum albumins is unchanged, while the proportion of fibrinogen is increased. There is much more fatty substance than normal in both the serum and the erythrocytes, but the lecithin is decreased both in the serum and in the total blood, although somewhat increased in the red cells. Cholesterin is decreased in both serum and corpuscles. In the ash, phosphoric acid, potassium, and iron are decreased, while calcium and magnesium are both increased. An apparent increase in sodium chloride exists, but it is only apparent, being the result of the increase in the proportion of plasma in the blood.

¹ Virchow's Arch., 1864 (29), 241.

² See Donath and Landsteiner, Zeit. klin. Med., 1905 (58), 173; Eason, Jour. Pathol. and Bact., 1906 (11), 203.

³ Zeit. klin. Med., 1902 (47), 302.

The decrease in hemoglobin is demonstrable chemically as well as microscopically, Becquerel and Rodier¹ having found the amount of iron in the total blood decreased in direct proportion to the apparent decrease in hemoglobin, which frequently falls to 30–40 per cent., and may drop to 20 per cent. or possibly less. Alkalinity, as determined by titration, is diminished in some cases, but generally remains nearly normal. The corpuscles are said to contain a larger proportion of water than normal, independent of the proportion of water present in the serum. Limbeck found their *isotonicity* (*i. e.*, the strength of NaCl necessary to prevent hemolysis) very low—about 0.38–0.4 per cent. NaCl.

Very few changes seem to occur in the organs of the body; the usual tendency to lay on fat, and the occurrence of fatty degeneration observed commonly in anemias, may be exhibited, and are correlated with Erben's observation of an increased fat content in the blood; but these changes are often absent. The hypoplasia of the aorta, upon which Virchow laid so much stress, is now considered to be of little or no significance. Thrombosis is a not infrequent complication of chlorosis,² and is probably favored by the increased fibrin-content of the blood and the tendency to fatty changes in the vessel-walls.

Studies of *nitrogenous metabolism* by Vannini³ showed practically no alterations except a slight retention of nitrogen.

Etiology.—As to the etiology of chlorosis, chemical findings indicate some possibilities and negative others, but decide nothing. That chlorosis does not depend upon a hemolytic poison is well established by the following facts: there is no free hemoglobin in the blood plasma, and even less iron in the serum ash than normal; lecithin and cholesterin, important products of disintegration of erythrocytes, are both decreased in the serum; hematogenous icterus does not occur, and the amount of pigments in the urine and feces is decreased.

Apparently, therefore, *hematogenesis* is at fault, particularly the formation of hemoglobin, since this is more deficient than is the total number of red corpuscles. The rapid improvement in the condition that follows the administration of iron would seem to indicate that a deficient supply of iron is the cause of chlorosis, but numerous objections exist to this hypothesis.

¹ For literature see Krehl, "Pathologische Physiologie," 1904, p. 137; Ewing, "Clinical Pathology of the Blood," 1901, p. 167; Kossler, *Cent. f. inn. Med.*, 1897 (18), 657.

² See Schweitzer, *Virchow's Arch.*, 1898 (152), 337, and Leichtenstern, *Münch. med. Woch.*, 1899 (46), 1603.

³ *Virchow's Arch.*, 1904 (176), 375.

Bunge advanced the idea that under normal conditions the only form of iron that can be absorbed is that which is combined with proteids, particularly nucleoproteids; iron administered in inorganic form, or as compounds with organic acids, he believed, can all be recovered from the feces, and, therefore, is not absorbed. He suggested that in chlorosis the iron taken with the ordinary food is precipitated in the intestines by sulphides or other products of intestinal putrefaction, and hence there results a deficiency in the amount of iron absorbed and available for the manufacture of hemoglobin. The inorganic iron given in chlorosis, Bunge believes, owes its efficiency to its saturating all of these sulphides so that the nucleoproteid-iron is not precipitated, and can, therefore, be absorbed. Many objections have been raised to Bunge's hypothesis, however, for competent observers have failed to find any abnormal putrefaction in chlorosis, and others have found that sulphide of iron itself gives good results in the treatment of chlorosis, while bismuth and other sulphur-binding substances are without effect. Furthermore, Bunge's contention that iron administered in medicinal form is not absorbed seems to have been completely disproved by several experimenters.¹

As a consequence of all these conflicting data we are at present completely in the dark as to the reason for that failure to properly manufacture hemoglobin which seems to be at the bottom of chlorosis. The hypothesis that iron and arsenic favor recovery by stimulating the hemogenetic tissues, which is urged by v. Noorden and others, is unsatisfactory in the extreme, and explains nothing. There is absolutely no question that administration of iron restores the composition of the blood to normal, usually quite rapidly, and this seems to leave as most probable the explanation that in some way an *iron starvation* is the fundamental cause of chlorosis. However, as Ewing says, any theory must be inadequate that fails to take into account the age of puberty, the female sex, and the function of menstruation.

PERNICIOUS ANEMIA

In contrast to chlorosis many evidences of hematology may be found in pernicious anemia, particularly the increased amounts of iron in the liver, spleen, and kidneys; hemoglobinemia and hemoglobinuria; increase in urobilin, and not infrequently icterus.

¹ Full review with bibliography by Abderhalden in his "Lehrbuch der physiol. Chemie," 1906, pp. 408-430. For literature on treatment of chlorosis see Romberg, Berl. klin. Woch., 1897 (34), 533.

Chemical Changes.—Erben's¹ analyses of the blood in pernicious anemia gave the following results: The proteids are decreased, both in the serum and in the blood as a whole; particularly in the latter, because of the great decrease in the number of corpuscles. The quantity of proteids in the individual corpuscles is increased, corresponding to their increased size. Fibrin is decreased in total amount, but relatively normal as compared with the total proteids; albumin is normal; serum globulin much decreased. The proportion of water is much increased, both in the serum and in the corpuscles. Fat is present in normal amounts; cholesterin is decreased, although in relatively normal quantities in the corpuscles. Lecithin is decreased in the total blood, but increased proportionately in the corpuscles. The total ash is increased, owing chiefly to an excessively large proportion of NaCl and a slight increase in calcium and magnesium; potassium and phosphoric acid are decreased because of the small number of corpuscles; but the serum itself contains more P_2O_5 and potassium than normal. Although the total iron is, of course, much decreased, there is iron in the serum (indicating hemolysis) and the proportion of iron in the corpuscles is increased; but as the amount of iron in the corpuscles is even greater than corresponds to the hemoglobin increase, it would seem that either the hemoglobin in pernicious anemia is very rich in iron, or that the corpuscles contain iron bound in some form other than hemoglobin.

The analyses of Rumpf² agree quite closely with those of Erben, and, taken jointly with other analyses in the literature, show the large proportion of water in the blood, the small amount of solids, the large amount of NaCl, and the decrease in potassium and iron. Rumpf also examined the brain, liver, heart, and spleen in one case. Water was found increased in the heart, decreased in the other organs, the solids not being decreased in any of the organs. There was little fat in any of the organs or in the blood, but NaCl was generally increased. The liver contained four or five times as much iron as normal; the spleen three or four times. Rumpf is inclined to lay great stress on the general poverty of the body in potassium, and suggests its therapeutic application. Syllaba³ found bilirubin and also free hemoglobin in the blood of seven patients. Schumm⁴ could find no proteoses or other evidences of proteid decomposition in the blood in a case of pernicious anemia.

v. Jaksch and also v. Limbeck⁵ have found some decrease in total alkalinity, which probably depends on the loss of proteids and their fixed alkali.⁶ The red corpuscles are very susceptible to hemolysis by lowering of osmotic pressure ("high isotonicity," equal to 0.54 per cent. NaCl—v. Limbeck). The specific gravity of the whole blood is, of course, decreased, being sometimes even lower than that of normal serum.

In six cases of pernicious anemia Stühlen⁷ found abundant iron in the liver and spleen microscopically, and less constantly in the kidneys and bone-marrow. Hunter⁸ gives the following results of analysis of the liver, kidney, and spleen for iron:

¹ Zeit. klin. Med., 1900 (40), 266.

² Berl. klin. Woch., 1901 (38), 477.

³ Abst. in Folia Hematol., 1904 (1), 283 and 589.

⁴ Hofmeister's Beitr., 1903 (4), 453.

⁵ "Klin. Pathol. des Blutes," Jena, 1896, p. 311.

⁶ See Brandenburg, Zeit. klin. Med., 1902 (45), 157.

⁷ Deut. Arch. klin. Med., 1895 (54), 248 (literature).

⁸ Lancet, 1903 (i), 283.

	Liver and kidney.	Spleen.
Pernicious anemia, seven cases average	0.360 per cent.	0.125 per cent.
Other conditions (with anemia), average	0.079 "	0.362 "
Healthy organs	0.084 "	0.090 "

Iron is also found in the hemolymph glands, sometimes more abundantly than in the spleen (Warthin¹).

Extensive studies on the *proteid metabolism* of pernicious anemia by Rosenquist² showed that there is a considerable destruction of tissue proteids, as indicated by nitrogen loss, but that at times nitrogen may be stored up for brief periods. At times there may also be an excessive elimination of purin nitrogen, indicating destruction of nuclear elements. In anemia due to *Bothriocephalus* quite similar changes were observed.

Hunter³ describes the condition of the urine in pernicious anemia, particularly with reference to the elimination of much "pathological urobilin,"⁴ which seems to be produced by intracellular destruction of hemoglobin. Iron also appears in the urine in considerable quantities.

Summary.—Putting together the above findings, we see that in pernicious anemia we have every evidence that excessive hemolysis is taking place, and the fact that continued poisoning by toluylendiamin⁵ and other hemolytic poisons, such as that of *Bothriocephalus*, may give rise to a condition resembling pernicious anemia very closely, indicates strongly that hemolytic poisons are the cause of pernicious anemia. Histological studies show the same thing, and, as Warthin⁶ says: "The hemolysis of pernicious anemia does not differ in kind from that occurring normally or in certain diseased conditions; the difference is one of degree only." The hemolysis seems to go on chiefly inside of phagocytic cells instead of in the blood, probably because the phagocytes pick up the corpuscles as soon as they have been injured by the hemolytic poisons. The origin and the nature of these hypothetical poisons have been sought in vain. Some authors have referred them to infections of unknown nature, occurring perhaps in the mouth and gastrointestinal tract (Hunter⁷), or to hemolytic products of intestinal putrefaction,⁸ or to faulty metabolism. Many others, with

¹ Amer. Jour. Med. Sci., 1902 (124), 674.

² Zeit. klin. Med., 1903 (49), 193 (literature.)

³ British Med. Jour., 1890 (ii), 1 and 81.

⁴ See also Mott, Lancet, 1890 (i), 287; and Syllaba, Abst. in Folia Hematol., 1904 (1), 283.

⁵ Syllaba, Hunter (*loc. cit.*).

⁶ *Loc. cit.*

⁷ Lancet, 1903 (1), 283.

⁸ See Külbs (Arch. exp. Path. u. Pharm., 1906 (55), 73), who found the intestinal contents of patients with chronic intestinal disorders to contain hemolytic substances of undetermined character.

Herter (Jour. Biol. Chem., 1906 (2), 1) suggests a relation between intestinal infection with *B. aerogenes capsulatus*, which produces hemolytic substances, and pernicious anemia.

perhaps the best of grounds, would ascribe pernicious anemia to a multiplicity of causes, which produce a protracted slight hemolysis that continues until the hematogenous organs give out, their exhaustion being perhaps hastened by the influence of the toxic substances in the blood; hematogenesis then becomes insufficient to replace the lost corpuscles, and the picture of pernicious anemia is established.¹

LEUKEMIA

In leukemia the chemical changes in the red corpuscles take a less prominent position, resembling either those of a secondary anemia or chlorosis, while the enormous number of leucocytes is the prominent feature and causes marked alterations in the composition of the blood. Large quantities of nucleoproteids and also of the intracellular enzymes are introduced into the blood by the excessive leucocytes. As the leucocytes are constantly breaking down, more or less of the products of their decomposition are present in the blood and appear in the urine. Because of the relatively slight metabolic activity of the lymphocytes the various chemical alterations are all less marked in lymphatic than in myelogenous leukemia.²

Chemistry of the Blood.—Considering the quantitative alterations in the constituents of the blood, we find the specific gravity lowered, but not so much as it would be in a simple anemia with equally low hemoglobin, for the loss of hemoglobin is partly compensated by the increase in leucocytes and their products. The serum shows but slight change in specific gravity, a slight decrease in proteids being compensated by an increase in the NaCl. The freezing-point of the blood is lowered (Cohn³), which is probably due to the increase in crystalloidal products of cellular decomposition. Erben⁴ found that in lymphatic leukemia the serum contains less cholesterin than normal, although the fat content may be rather high. Calcium is frequently found increased, probably because of destruction of the bone tissue. In the red corpuscles the proportion of iron is decreased as is also that of the cholesterin, that of the lecithin being somewhat increased. The total amount of potassium and iron in the blood is decreased, but the P_2O_5 in the ash is increased because of the large amount of nucleoproteid in the blood. A number of the earlier writers describe a decreased alkalescence which probably is due to the deficiency in the fixed alkali of the proteids. Scherer and others have reported the finding of lactic, formic, and acetic acids in leukemic blood.

¹ See also Bunting, Johns Hopkins Hosp. Bull., 1905 (16), 222.

² Stern and Eppenstein have observed that the striking proteolytic power of the leucocytes from the blood in myelogenous leukemia is not shown by the leucocytes in lymphatic leukemia (Sitz. d. Schles. Ges. f. vaterländ. Cultur, June 29, 1906).

³ Mitteil. aus dem Grenzgeb. Med. u. Chir., 1906 (15), H. 1.

⁴ Zeit. klin. Med., 1900 (40), 282.

The poor coagulation of leukemic blood has been long known, but the reason for it has not yet been ascertained. Some investigators have reported a deficiency in fibrin, while others have found it increased. More recent reports, however, indicate that there is no marked change in either the amount of fibrinogen or of the fibrin-ferments. Erben¹ found a normal amount of fibrin in the blood in lymphatic leukemia; and in three cases of myelogenous and one of lymphatic leukemia, Pfeiffer² found the amount of fibrinogen nearly normal. This is quite remarkable in view of the fact that in ordinary forms of leucocytosis both the amount of fibrinogen and the rapidity of clotting are increased. It is, therefore, extremely difficult to understand the poor coagulability of leukemic blood.

Decomposition Products.—Of particular interest is the finding in the blood of decomposition products of the leucocytes, which are probably produced by autolysis of the leucocytes. Normal leucocytes are rich in autolytic enzymes, which under ordinary circumstances seem to be held in check by the anti-enzymes of the blood. In leukemia this anti-enzyme action seems to be insufficient to prevent leucocytic autolysis, for even in freshly drawn blood proteoses (or at least non-coagulable proteids) may be present.³ According to Erben, this is true only of myelogenous leukemia, the fresh blood in lymphatic leukemia not only being free from non-coagulable proteid, but furthermore this product of proteolysis does not soon develop when the blood is kept aseptically at incubator temperature. This is, of course, what one would expect in view of the well-known enzyme-richness of the polymorphonuclear leucocytes and the scarcity of enzymes in lymphocytes. Erben states that the neutrophile cells seem to be the chief source of proteoses, since their granules soon disappear in blood that is undergoing autolysis, whereas the eosinophiles preserve their granules well, and true proteoses are not present in blood rich in mast cells (*i. e.*, myeloma). Schumm⁴ found in the blood in a case of myelogenous leukemia several varieties of proteoses, most abundant being the so-called deuterio-albumose; in another he also found peptone, leucin, and tyrosin. In addition he demonstrated the autolytic nature of the changes that occur in leukemic blood after death (see also "Autolysis in Leukemia," Chap. iii). Most observers have failed to find *albumose* in the urine in

¹ *Loc. cit.*

² *Cent. f. inn. Med.*, 1904 (25), 809.

³ For literature see Erben, *Zeit. f. Heilk. (Int. Med. Abt.)*, 1903 (24), 70.

⁴ Hofmeister's *Beitr.*, 1903 (4), 442; *Deut. med. Woch.*, 1905 (31), 183.

leukemia; Askanazy¹ reports finding what he describes as Bence-Jones albumose in one case of lymphatic leukemia, but this was afterward found to be a case of multiple myeloma.² Kolisch and Burián³ not only found nucleoproteid constantly, and albumose frequently, but in one case of lymphatic leukemia they found histon in the urine, which undoubtedly came from nucleoproteid decomposition.

Proteid Metabolism.—Stejskal and Erben⁴ studied the metabolism of a case of myelogenous and of a case of lymphatic leukemia, and found the nitrogen loss much greater in the myelogenous form, although food-absorption was better than in the lymphatic; they consider that proteid-destroying forces are at work in myelogenous leukemia, similar to those of cancer cachexia, so that nitrogenous equilibrium cannot be attained.

As the most characteristic products of decomposition of nucleoproteids are the purin bases, one would also expect to find them present in leukemia, and early writers mention the finding of purin bases and uric acid in the blood and spleen. The urinary findings in this respect have been very variable. Ebstein⁵ observed the complication of leukemia with gout, which he considered a coincidence, and also noted uric-acid concretions in the urinary passages in four cases. Numerous other authors have described increased uric-acid elimination, while some have observed increase in the purin bases, either with or without uric-acid increase. Magnus-Levy⁶ observed a particularly large uric-acid output in acute leukemias, but also found that the relation between the number of leucocytes and the uric acid is extremely variable. Sometimes the nitrogen loss is very great—even as much as 20 gm. per day—and, corresponding with the destruction of nucleoproteids and the resulting uric-acid formation, phosphoric-acid excretion is often greatly increased—even up to 15 gm. per day. On the other hand, the results obtained by many other writers have been in every respect extremely variable; some have found no increase in uric acid, some even report a decrease; likewise the P_2O_5 has been found even less than normal. For example, in a carefully studied case of lymphatic leukemia, Henderson and Edwards⁷ found during six months no excessive excretion of uric acid or phosphoric

¹ Deut. Arch. klin. Med., 1900 (68), 34.

² See "Myeloma," Chap. xvii.

³ Zeit. klin. Med., 1896 (29), 374 (literature on albuminuria in leukemia).

⁴ Zeit. f. klin. Med., 1900 (39), 151.

⁵ For literature see résumé by Walz in Cent. f. Pathol., 1901 (12), 985.

⁶ Virchow's Arch., 1898 (152), 107.

⁷ Amer. Jour. of Physiol., 1903 (9), 417.

acid. Zalesky and Erben found likewise no considerable increase in the uric acid in lymphatic leukemia, but in myelogenous leukemia the uric acid was much increased; on the other hand, the amount of elimination of purin bases was reversed in the two forms, and creatin was decreased in both. Lipstein¹ found no excessive elimination of amino-acids even in myelogenous leukemia. An increase in calcium is quite constantly observed, and attributed to the bone destruction² occurring in this disease.

Undoubtedly these variations in results depend upon the known fluctuations in the course of the pathological processes of leukemia; the number of leucocytes, the size of the lymphatic organs, and the general condition of the patient all vary greatly from time to time, often with remarkable rapidity, and the excretion of products of metabolic activity must vary likewise. It can hardly be questioned that the enormous increase in the amount of lymphoid tissue in the body and blood must give rise to a greatly increased nuclein catabolism, with consequent appearance of its products (uric acid, purin bases, and phosphoric acid) in the urine. This seems to be well demonstrated by the increased elimination of uric acid and purin bases, together with a general increase in the nitrogen output that has been frequently observed following the therapeutic use of *x*-rays in leukemia, which is attributed to the increased autolysis that *x*-rays are known to produce. Lipstein³ also found an excessive elimination of amino-acids in the urine of leukemic patients treated by *x*-rays.⁴ According to Curschmann and Gaupp,⁵ the blood of leukemic patients who have been exposed to *x*-rays contains a specific *leucocytotoxin*, which may be produced by a process of autoimmunization against the leucocytic substance set free by the disintegrated leucocytes. Capps and Smith⁶ have obtained similar results.

Charcot's crystals (also called Charcot-Leyden and Charcot-Neumann crystals) represent a peculiar and striking product of nuclear destruction that has frequently been found associated with leukemia.

¹ *Loc. cit. inf.*

² Stejskal and Erben, *loc. cit.*

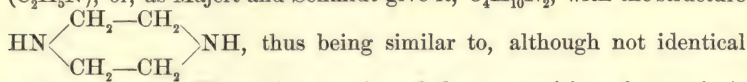
³ Hofmeister's Beitr., 1905 (7), 527.

⁴ Literature on effects of *x*-rays in leukemia, see Arneth, Berl. klin. Woch., 1905 (42), 1204; Musser and Edsall, Univ. of Penn. Med. Bull., 1905 (18), 174; Rosenberger, Münch. med. Woch., 1906 (53), 209; Williams, Biochem. Jour., 1906 (1), 249; Lossen and Morawitz, Deut. Arch. klin. Med., 1905 (83), 288; Königer, Deut. Arch. klin. Med., 1906 (87), 31.

⁵ Münch. med. Woch., 1905 (52), 2409.

⁶ Trans. Chicago Path. Soc., 1905 (6), 371; see also Klieneberger u. Zoepfritz, Münch. med. Woch., 1906 (53), No. 18; Milchner u. Wolff, Berl. klin. Woch., 1906 (43), No. 23.

These crystals were first observed by Robin¹ (1853) in leukemic tissues, but have been named after Charcot, who, with Robin, described their properties. They were described by Charcot as colorless, refractile, elongated octahedra; insoluble in alcohol, ether, and glycerin; soluble in hot water, acids, and alkalis; size variable, from 0.016 by 0.005 mm. up. These crystals have been found not only in the tissues and blood of cadavers, but also occasionally in the freshly drawn blood of leukemics. Poehl² believes them to be the same as Böttcher's *spermin crystals*, and derived from decomposed nucleins. Schreiner considers that these spermin crystals are phosphoric acid salts of spermin (C_2H_5N), or, as Majert and Schmidt give it, $C_4H_{10}N_2$, with the structure



with, *piperazin*.³ The entire question of the composition of spermin is still unsettled,³ however; and it is probable, furthermore, that the crystals found in leukemia are not identical with the crystals observed in semen.

Crystals that appear similar are also found in asthmatic sputum, empyema, and ascites fluid, bone-marrow, and tumors, and it has been suggested that they are derived from or related to the oxyphile granules of the eosinophiles.⁴ This view implies an agreement with Gumprecht's opinion that the crystals seen in bone-marrow, asthmatic sputum, etc., are not spermin, but of proteid nature. As can be seen, the nature and significance of Charcot's crystals are, at the present time, quite undetermined.

Summary.—The chemical changes observed in leukemia depend upon the excessive quantity of leucocytes and lymphoid tissue, which undergo processes of disintegration at irregular intervals, with the result that the products of nucleoproteid destruction (uric acid, purin bases, and phosphoric acid) appear in the urine in increased quantities. As the large neutrophiles contain abundant autolytic enzymes, the products of cell autolysis (proteoses, amino-acids, and products of nucleoproteid destruction) may appear at times in the urine and in the blood; because of the small amount of such enzymes in the lymphocytes, these changes are all much less marked in lymphatic leukemia. Charcot's crystals, which are perhaps derived from leucocytic nucleoproteids, may be found in the blood and tissues. The changes in the red cells are chiefly those of a secondary anemia, with occasionally some chlorotic features. The chemical findings of leukemia throw no light whatever upon the cause of the disease.

Hodgkin's disease (*pseudo-leukemia*) shows only the evidences

¹ Earlier literature given by Ewing, "Clinical Path. of Blood," 1901, p. 218; and by v. Limbeck, "Clinical Path. des Blutes," 1896, p. 318.

² Deut. med. Woch., 1895 (21), 475.

³ Literature, see Hammarsten, Amer. Transl., 1904, p. 420.

⁴ Literature, see Floderer, Wien. klin. Woch., 1903 (16), 276; Predtetschensky, Zeit. klin. Med., 1906 (59), 29.

of a secondary anemia, without the chemical changes of either leukemia or pernicious anemia. There seems to have been little study of the chemical processes of this disease. Moraczewski¹ reports a study of metabolism in one case, which showed some retention of nitrogen and calcium, with little change in the phosphorus and purin bases in the urine.

HYPEREMIA

ACTIVE HYPEREMIA

This condition is associated with but few chemical changes. Certain chemicals may cause active hyperemia; some locally, as in the case of irritants, such as alcohol, ether, ammonia, mustard, etc., which act either by producing a local vasodilator stimulus or by paralyzing the vasoconstrictors. Other substances may produce active hyperemia in special vascular areas, *e. g.*, cantharides causes active hyperemia in the kidneys, probably because of its elimination through these organs; pilocarpin causes active hyperemia in the salivary glands and skin, which is associated with increased function. In general, functional activity is associated with active hyperemia, and Gaskell² has suggested that this is due to atonicity of the vascular muscle, the result of decreased alkalinity of the lymph flowing away from the active organ along the vessel-walls, it having been found that alkalis cause a tonic contraction and acids an atonic dilatation of arterial muscle.

Pathological active hyperemia is seldom of long enough duration to lead to any alterations in the tissues in which it occurs. The blood itself remains unchanged, except that the venous blood going from the part contains much less CO₂ and more oxygen than usual, because more oxygen is brought to the tissues than can be used.

PASSIVE HYPEREMIA

Passive hyperemia is almost equally unassociated with chemical changes, especially in its etiology, which depends almost solely upon mechanical factors. Some chemical alterations result, however, from the changes in the stagnating blood, which may, if the obstruction to outflow is severe, become of venous character in the capillaries of the congested area. Oxidation in the tissues is, therefore, impaired, and some fatty changes may result, *e. g.*, in the center of congested liver lobules. Waste

¹ Virchow's Arch., 1898 (151), 22.

² Quoted by Lazarus-Barlow, "Manual of General Pathology," 1904, p. 126.

products accumulate, and possibly noxious products of metabolism are formed under lack of oxidation; either from these causes or solely from pressure and lack of nutrition there is a tendency to atrophy of the more specialized parenchymatous cells, and a proliferation of connective tissues. The atrophy of parenchyma is seen particularly in the liver, the increase of connective tissue in the spleen.¹ In the kidney neither atrophy nor stroma proliferations are pronounced, but the renal function is greatly impaired, since it depends upon the amount and quality of the blood brought to the kidney. Whether connective-tissue proliferation in hyperemia depends upon overnutrition or upon irritation by waste-products, or is compensatory to parenchymatous atrophy, may be looked upon as still an open question. Probably only the first two factors apply to the connective-tissue growth observed in the congested spleen, the clubbing of the fingers in congenital heart disease, or the thickening of the subcutaneous tissues in passive congestion of the lower extremities. The edema of passive congestion seems to result partly from mechanical forces and partly from the high osmotic pressure that develops in the underoxygenated tissues (see "Edema," Chap. xii).

Changes in the Blood.—Venous blood differs from arterial, not only in its increased load of CO_2 and other waste-products, but also in other ways. Venous blood generally clots less readily than arterial blood.² It contains more diffusible alkali because the CO_2 combines with and tears away part of the bases that are held by the proteids, especially in the corpuscles, and so alkaline carbonates are formed and enter the plasma. Blood from the jugular vein on this account contains 20–25 per cent. more diffusible alkali than carotid blood (Hamburger³). Since the bactericidal power of the blood has been shown to increase directly with the alkalinity, this property may be of importance in pathology. For example, the relative infrequency of infections in the right side of the heart may not depend solely upon lessened liability to endocardial damage, as generally considered, but is possibly due in part to the greater bactericidal power of venous blood. The same property probably explains the favorable results obtained in the treatment of local infections by artificially produced passive congestion.⁴

¹ See Christian, *Jour. Amer. Med. Assoc.*, 1905 (45), 1615.

² Vierordt (*Arch. f. Heilk.*, 1878 (19), 193) found coagulation faster in the blood in passive congestion than in normal venous blood; but Hasebrock (*Zeit. f. Biol.*, 1882 (18), 41) found that if the stasis is protracted, the coagulation becomes delayed because of the excess of CO_2 .

³ Virchow's *Arch.*, 1899 (156), 329; also, "Osmotischer Druck und Ionenlehre," 1902, p. 280.

⁴ See Bier, "Hyperämie als Heilmittel," Leipzig, 1903.

v. Fodor¹ found that animals surviving infections showed an increased blood alkalinity, whereas in those that died, the alkalinity was decreased; also, he found the resistance increased by intravenous injections of alkalies. Other observers² have noted a decrease in resistance after injecting acids into the blood. According to Calabrese, the alkalinity of the blood increases in immunization of animals against toxins, while Cantani found the injection of toxin followed by a decrease in alkalinity. Hamburger has shown that the bactericidal power of the blood may be increased *in vitro* by shaking it with CO₂, as a result of the increased alkalinity, aided, perhaps, by some slight bactericidal power of the CO₂ itself; he also found the blood more strongly bactericidal in venous congestion than normally, and the lymph from a congested part was also found more strongly bactericidal than normal lymph. Hamburger³ has also found, however, that chemotaxis is, if anything, slightly decreased under the influence of CO₂, as also is phagocytosis; large amounts of CO₂ may reduce the phagocytic power for coal particles by 25–50 per cent. Hamburger's results as to the bactericidal power of human blood in venous stasis have been more recently confirmed by Laqueur.⁴

The blood in the veins and capillaries in passive congestion is generally richer in corpuscles than normal, perhaps because of some loss of water,⁵ although this is not constant, applying particularly to more recent or more local processes; in long-continued stasis, as in congenital heart disease, the blood may be diluted.⁶ In the concentrated blood of passive congestion the corpuscles may number six to eight millions per cubic millimeter, while the concentration of the solids of the serum may be at the same time reduced (Krehl). The viscosity of such blood is higher than that of normal blood.⁷

THROMBOSIS

The chemistry of thrombosis in most respects resolves itself into the chemistry of fibrin formation, a subject which is so extensively considered in most treatises on physiological chemistry and physiology that it does not seem desirable to give here

¹ Cent. f. Bakt., 1890 (7), 753.

² Literature, see Hamburger (*loc. cit.*), p. 281.

³ Virchow's Arch., 1899 (156), 329.

⁴ Zeit. exp. Path. u. Therap., 1905 (1), 670.

⁵ Grawitz, Deut. Arch. f. klin. Med., 1895 (54), 588.

⁶ See Krehl, "Pathologische Physiologie," 1904, p. 201.

⁷ Determann, Zeit. klin. Med., 1906 (59), H. 2–4.

anything more than the essential principles involved in the clotting of the blood, as now understood, as an introduction to the consideration of the same process as it occurs under pathological conditions. In spite of innumerable investigations, our knowledge of the actual participants and processes involved in the formation of fibrin is in a very unsatisfactory and fragmentary state. Some facts seem well established, however, and we have a general idea of the subject that may be applied with advantage to the consideration of thrombosis.

FIBRIN FORMATION¹

Several different substances seem to be concerned in the formation of fibrin, of which the first of importance is its antecedent, *fibrinogen*. Fibrinogen is a simple proteid, related to the globulins, and differing chiefly in its ready coagulability, not only by fibrin ferment, but also by heat, salts, and other coagulating agencies. By itself, however, it shows no tendency to coagulate spontaneously. According to Mathews,² fibrinogen is formed chiefly in the intestinal walls from the leucocytes. Acted upon by the fibrin-ferment, it yields the characteristic insoluble proteid, fibrin; probably the change consists in a cleavage of the fibrinogen molecule into fibrin and a small amount of a soluble proteid. Fibrin resembles in its insolubility the proteids coagulated by heat, alcohol, etc., but when kept aseptically for some time, it becomes again dissolved; this process of *fibrinolysis* probably depends upon proteolytic enzymes which fibrin, in common with other substances of similar physical nature, has the property of dragging out of solution and holding firmly. Undoubtedly entangled leucocytes are also an important factor in the fibrinolysis.³

Theories of Fibrin Formation.—The great problem is the nature and the place and manner of origin of the fibrin-forming enzyme, generally called *fibrin-ferment* (also *plasmase* and *coagulin*). It has been conclusively shown that the agent causing the formation of fibrin from fibrinogen is a true enzyme, but, as with the other enzymes, it is not known whether fibrin-ferment is a nucleoproteid (Pekelharing) or any other sort of a proteid. The best known and most fundamental theory of the origin and nature of fibrin-ferment is that of Alexander Schmidt, which may be briefly described as follows: The ferment, Schmidt believes, exists in the plasma in an inactive (*prozyme* or *zymogen*) form, which he calls *prothrombin*. Upon disintegration of the leucocytes there is set free a substance, which, acting upon the prothrombin, converts it into the active thrombin; this activating agent Schmidt designates as the *zymoplastic* substance. With various modifications this theory stands

¹ For literature and full discussion see Hammarsten's *Physiological Chemistry*; more recent literature by Morawitz, *Ergebnisse der Physiol.*, Abt. 1, 1904 (4), 307; and Blum, *Cent. f. Path.*, 1904 (15), 385. Résumé of recent work by Leo Loeb, *Medical News*, 1905 (86), 577.

² *Amer. Jour. Physiol.*, 1899 (3), 53; a different view is held by Doyon, Merel and Kareff (*Jour. de physiol.*, 1906 (8), 783).

³ See Morawitz, *loc. cit.*; also Rulot, *Arch. internat. d. Physiol.*, 1904 (1), 152.

to the present day as best explaining the facts concerning fibrin formation with which we are familiar.

It having been shown that calcium facilitates the formation of fibrin, Pekelharing advanced the idea that the prothrombin does not exist in the plasma, but is liberated from the leucocytes, and, combining with the calcium of the plasma, forms the thrombin. Morawitz considers three substances necessary for the formation of thrombin: (1) the *prothrombin* or *thrombogen*, which he believes originates in the blood-plates; (2) the *zymoplastic substance* or *thrombokinas*, which is liberated from the leucocytes into the plasma; (3) calcium salts. The chief differences, therefore, are not concerning the nature of the thrombin, but the manner of its origin, and whether the prothrombin arises from the leucocytes, the plasma, or the platelets. It seems quite probable, however, that prothrombin may occur in all these constituents of the blood; since coagulation occurs in the lymph, which contains no platelets, the prothrombin evidently is not derived solely from these elements. It will not serve our purpose, however, to go further into the hypotheses and disputes over these questions, which are detailed more fully in the literature previously cited.

The question has been raised as to whether the leucocytes secrete their fibrin-forming constituent (be it thrombokinas or prothrombin is a matter of minor importance to the pathologist) or liberate it only after their disintegration. So far as pathological processes go, the latter seems to be the case, the disintegration apparently occurring whenever the leucocytes come in contact with a foreign body or with dead and injured tissues.¹

Tissue Coagulins.—Among the other points that are of importance in pathological conditions is the fact that not only leucocytes, but also tissue-cells, can liberate fibrin-forming substances (*coagulins* is the non-committal term applied by Loeb). These *tissue-coagulins* are present in tissue extracts and are liberated whenever the tissues are injured; muscle is rich in coagulin, as are also the liver and kidney, and, which is particularly important, the blood-vessel wall (L. Loeb). Pieces of these tissues placed in contact with fibrinogen solution will bring about prompt clotting. Another important fact is that the coagulins, whether derived from leucocytes or from the tissues, have a certain degree of specificity—that is, they act solely or most rapidly with fibrinogen of blood of the species from which they are obtained.² In some instances this specificity is absolute, but more generally (particularly in the mammalia) it is only relative. Loeb also found that the amount of tissue coagulin was not decreased in organs altered by phosphorus-poisoning, although during experimental autolysis the coagulins disappear. When tissue coagulins and blood coagulins act together, the effect is greater than the sum of their independent actions, indicating the probability that they combine in some way to produce a particularly active coagulin. The blood coagulins are quite different from the tissue coagulins in many important respects, and the coagulins cannot be looked upon as a single substance of different origins.

Blood-platelets.³—It is still undetermined just what part the platelets

¹ See L. Loeb, *loc. cit.*

² Leo Loeb, *Univ. of Penn. Med. Bull.*, 1904 (16), 382; Muraschew, *Deut. Arch. klin. Med.*, 1904 (80), 187.

³ Earlier literature, see Schwalbe, *Ergebnisse der Pathol.*, 1902 (8), 150; and Löwit, *ibid.*, 1895 (2), 642.

play in coagulation. The well-known observation that in thrombosis the fibrin is often first formed about masses of platelets clinging to the wall of the vessel indicates that they participate in the process, and Bizzozero and others have maintained that the platelets and not the leucocytes are the source of the prothrombin. Numerous studies on the relation of the platelets to disease conditions have indicated a certain parallelism between their number and the tendency to coagulation observed in the various diseases (Welch). Pratt,¹ however, found that the number of platelets bore no relation to the coagulability of the blood; and lymph, which is free from platelets, will coagulate. It is, therefore, probable that platelets are one, but not the sole, source of thrombin. Kemp² concludes, from a thorough review of the subject, that the blood-platelets are usually normal or subnormal in number during acute infectious diseases, but increase rapidly if the disease terminates by crisis; in pernicious anemia the number is always greatly diminished, although in secondary anemias they may sometimes be increased; in *purpura hæmorrhagica* the number of plates is enormously diminished, which is perhaps related to the slowness of the clotting of the blood in this condition.

Calcium Salts.—The exact significance of calcium in fibrin formation is also unsettled.³ Blood from which the calcium has been precipitated will not coagulate, and the addition of calcium salts will promptly cause it to do so; furthermore, the coagulability of the blood, whether normal or below normal, may be greatly increased by the administration of calcium salts to the subject by mouth (Wright⁴). The various hypotheses advanced to explain the way in which calcium influences the clotting process are not in agreement. Perhaps the most probable hypothesis is that the calcium ions are necessary for the transformation of prothrombin into thrombin (Pekelharing, Hammarsten, Morawitz), the thrombin consisting of a compound of prothrombin, calcium salts, and thrombo-kinase.

Modification of Coagulability.—Another important matter for consideration is the effect of various substances in modifying the rate or completeness of the coagulation of the blood. In the first place, we have the well-known fact that if blood is drawn into a glass vessel well coated with oil or vaseline, through a cannula similarly protected, no coagulation will take place; but if any unoiled foreign substance enters, even particles of dust, coagulation begins at once. The explanation is that the leucocytes do not liberate their coagulating substances until they have been injured by contact with some foreign body, and the experiment proves the importance of this action of the leucocytes, as well as explaining why the blood does not coagulate during life. The classical experiment of the ligation of a vein without injury to the endothelium, which permits the blood

¹ Jour. of Med. Research, 1903 (10), 120.

² Jour. Amer. Med. Assoc., 1906 (46), 1022.

³ See Hammarsten, Zeit. physiol. Chem., 1896 (22), 333.

⁴ British Med. Jour., 1894 (ii), 57; also Boggs, Deut. Arch. klin. Med., 1904 (79), 539.

to remain stagnant for a long period without clotting, depends upon the same fact, namely, that normal endothelium neither liberates coagulin itself nor injures the leucocytes so that they disintegrate. Loeb recalls the observation of Overton that lipoids are important constituents of the cell membranes, and suggests a similarity between the vessel lining and the oiled cannula. The suggestion that the vessel walls contain an *anti-coagulin* could not be confirmed by Loeb. Since leucocytes are constantly undergoing disintegration in the blood and tissues under normal conditions, it might be asked why they do not cause clotting then and there. In explanation Loeb advances his observation that the coagulins are destroyed during cell autolysis, and suggests that when leucocytes normally disintegrate, the coagulins are first destroyed by autolysis. It has also been shown that the cells and serum contain substances which inhibit or prevent coagulation, and it is possible that these play an important part under normal conditions in preventing coagulation by products of cell disintegration, much as other anti-enzymes are supposed to act in preventing autodigestion of living cells.

Coagulation of drawn blood may be *retarded* experimentally by removal of the calcium by precipitation as oxalate, fluoride, etc.; also by diminishing the oxygen and increasing the CO₂, by addition of solutions of neutral salts in large amounts, by diluting greatly with water, or by keeping the blood cold. Coagulation may be hastened by moderate heat, by whipping, exposure to air, by contact with much foreign matter, and by the addition of watery extracts from many different tissues and organs.¹ Of particular interest pathologically is the retardation of coagulation that follows injections of proteoses (the so-called "peptone" solution) and also by various other proteid-containing solutions, such as organ extracts, bacterial toxins, snake venoms, eel serum, extract of leeches or of *Uncinaria*, impure nucleoproteid solutions, or solutions of various colloids. Most of these substances (*e. g.*, peptone, eel serum) cause reduction of coagulability when injected into animals, and are without effect on blood removed from the body. A few, however, prevent coagulation of drawn blood (snake venom, extract of leeches). When substances of the first class are injected in sufficient quantities, there occurs first a period of accelerated coagulation which may, particularly in the case of organ extracts, cause prompt death from intravascular clotting; if the animal survives, there follows a period of decrease or total inhibition

¹ See Conradi, Hofmeister's Beitr., 1901 (1), 136.

of coagulability of the blood, both within the vessels and after removal from the body. The first period of increased coagulability undoubtedly depends upon the formation of a large amount of fibrin-ferment, but it has not yet been satisfactorily explained how the inhibition of coagulation is produced. Apparently the fibrin-ferment formed at first is rapidly destroyed, but it is thought by some that it is converted into a substance that neutralizes the fibrin-ferment that may be formed later, or that a true anticoagulin is formed. It is also among the possibilities that all the available prothrombin or thrombokinas is used up during the first stage of acceleration. As before mentioned, the blood and tissues contain substances that inhibit coagulation, and it may be that these are secreted in excessive amounts. It has been found that in animals deprived of the liver no coagulation-inhibiting substances are formed in the blood after injection of proteoses, hence Delezenne believes that the substances of this class act by causing a destruction of leucocytes, thus liberating a substance which increases coagulation and also another substance retarding coagulation; the first of these is destroyed by the liver, leaving the retarding substance to act unopposed.¹ Leech extract (*hirudin*) prevents clotting by means of an antiferment action, combining with the thrombin. Snake venom, however, acts upon the thrombokinas (Morawitz).

Coagulability of the Blood in Disease.—In disease the alterations in the coagulability of the blood depend upon much the same factors. The high coagulability in lobar pneumonia is undoubtedly caused, at least in part, by an excessive formation of fibrin-ferment through the extensive disintegration of leucocytes. In all conditions associated with suppuration and leucocytosis the amount of fibrinogen is also increased. The fluidity of the blood in septicemia is probably dependent upon the appearance of the coagulation-inhibiting phase that follows the action of the products of cell destruction, including among them proteoses. In this connection should be mentioned the observation of Conradi,² who found that among the products of autolysis is a coagulation-inhibiting substance which is not destroyed by heat, diffuses readily, and in general behaves unlike the proteids. This or similar substances may well play a part in affecting coagulation in infectious diseases. It may also be mentioned that animals soon acquire an immunity against

¹ The manner in which gelatin injections cause an increase in the blood coagulability is not yet understood (see Boggs, Deut. Arch. klin. Med., 1904 (79), 539).

² Hofmeister's Beitr., 1901 (1), 137.

proteoses, so that their inhibiting influence is no longer shown. This corresponds to the observation of Kanthack¹ that immune serum against venom neutralizes very effectively the anticoagulating principle of venom; an amount of antiserum altogether insufficient to neutralize the toxic properties of venom will neutralize its property of preventing clotting. The bacterial products may also modify coagulation, and L. Loeb² has found that different organisms are unequally effective in this respect, *Staphylococcus aureus* being much more powerful in causing coagulation than any others tested; typhoid, diphtheria, tubercle, and xerosis bacilli and streptococci being without any apparent effect, while pyocyaneus, prodigiosus, and colon bacilli occupy an intermediate position. Furthermore, after the organisms are killed by boiling, this effect is greatly reduced, showing that it does not depend merely upon the mechanical action of the bacteria, but probably upon bacterial products contained in the culture-media.

After phosphorus-poisoning the blood may become non-coagulable, which, Jacoby³ found, was due to an absence of fibrinogen in the blood; this Jacoby attributed to a fibrinogen-destroying ferment in the liver. As yet this is the only known example of non-coagulability due to absence of fibrinogen, with the exception of Doyon's⁴ similar finding in chloroform necrosis of the liver. In other instances of decreased coagulability the fibrinogen is present, generally in normal amounts. After death the blood becomes incoagulable because the fibrinogen is destroyed through a process similar to that of fibrinolysis;⁵ this fibrinolysis may be complete as early as ten hours after death. The other proteids of the blood do not seem to be correspondingly attacked. Thrombokinase is also scanty in cadaver blood, but there seem to be no coagulation-inhibiting substances present.

Pfeiffer⁶ estimated the fibrin content of the blood in disease, and found it increased in diseases with leucocytosis (pneumonia, rheumatism, erysipelas, scarlet fever), except leukemia, where it was normal; in diseases without leucocytosis (typhoid, malaria, nephritis), the fibrin was normal in amount. Stassano and Billon⁷ have, furthermore, shown that the amount of fibrin-fer-

¹ Cited by Lazarus-Barlow, p. 141.

² Jour. Med. Research, 1903 (10), 407.

³ Zeit. physiol. Chem., 1900 (30), 175; also Doyon *et al.*, Compt. Rend. Soc. Biol., 1905 (58), 493.

⁴ Compt. Rend. Soc. Biol., 1905 (58), 704.

⁵ Morawitz, Hofmeister's Beitr., 1906 (8), 1.

⁶ Zeit. klin. Med., 1897 (33), 214; Cent. f. inn. Med., 1898 (19), 1.

⁷ Compt. Rend. Soc. Biol., 1903 (55), 511.

ment varies directly with the number of leucocytes in the blood. Kollmann¹ found an increase in the fibrin in eclampsia, which Lewinski² could not substantiate. In experimental infections of animals Langstein and Mayer³ found a specific increase in pneumococcus sepsis, which undoubtedly bears an important relation both to the characteristic fibrinous nature of the alveolar exudate in pneumonia, and the striking amount of fibrin found in pneumococcus pleuritis, peritonitis, etc. Mathews⁴ found an increase in the fibrin with all experimental suppurations.

The **coagulation time** of the blood may be determined experimentally by methods devised by Vierordt,⁵ A. E. Wright,⁶ and by Brodie and Russell,⁷ the last named being considered the best by Murphy and Gould.⁸ The average time of coagulation is between three and six minutes. In jaundice a delayed coagulation time has generally been observed, but was not constantly found by Murphy and Gould.

THE FORMATION OF THROMBI

If we apply the facts brought out in the preceding discussion relative to the factors in the coagulation of blood, to the manner and conditions under which thrombi are formed in the circulating blood, we find explanations for many of the features of thrombosis. Welch⁹ describes the steps in the formation of a thrombus after injury to the vessel-wall, as follows: First, there is an accumulation of blood-platelets adhering to the wall at the point of injury. Leucocytes, which may be present in small numbers at the beginning, rapidly increase in number, collecting at the margins of the platelet masses and between them. Not until the leucocytes have accumulated does the fibrin appear. As Welch remarks, these findings afford no conclusive evidence as to whether fibrin-ferment is formed from the leucocytes or from the platelets, but since the fibrin does not appear until after the leucocytes have accumulated, and also since small thrombi may consist solely of platelets without fibrin, it seems probable that the leucocytes must be looked upon as the chief source of the ferment. Sometimes small clots may form without the apparent participation of either platelets or leucocytes. These purely fibrinous thrombi seem to start from

¹ Cent. f. Gynak., 1897 (21), 341.

² Pflüger's Arch., 1903 (100), 611.

³ Hofmeister's Beitr., 1903 (5), 69.

⁴ Amer. Jour. Physiol., 1899 (3), 53.

⁵ Arch. f. Heilk., 1878 (19), 193.

⁶ Brit. Med. Jour., 1894 (i), 237.

⁷ Jour. of Physiol., 1897 (21), 403.

⁸ Boston Med. and Surg. Jour., 1904 (151), 45.

⁹ Albutt System, vol. 6, complete discussion of the general features of thrombosis; also see Jores, Ergebnisse der Pathol., 1888 (5), 1.

injured endothelial cells, particularly in inflammatory conditions, such as pneumonic lungs, and give the impression that the coagulin is derived from the endothelial cells.

The process of clotting in the stoppage of hemorrhage offers some differences from intravascular clotting, in that the coagulins of the tissue-cells also come into play. It is rather difficult to determine how much of the coagulation depends on these, and how much on the coagulins of the leucocytes, for the same conditions that favor liberation of tissue coagulins, *i. e.*, much laceration and destruction of the tissue, also favor the disintegration of leucocytes by offering large areas of surface for contact. Loeb is of the opinion, however, that of the two, the latter factor is the more important. It may be recalled that the joint action of tissue and blood coagulins is greater than the sum of their individual actions, which also must be an important factor in causing clotting in bleeding wounds.

As to the relative importance of stagnation and vessel injury in producing thrombosis, we know that total stasis in an uninjured vessel may not result in thrombosis, and, on the other hand, extensive injury or large calcified plaques in the intima of the aorta may also cause no thrombosis because of the rapidity of the blood flow; and, furthermore, clotting may occur even in intact vessels under the influence of substances liberating fibrin-ferment in the blood; *e. g.*, snake venoms, nucleoproteid injections, and possibly in disease. Presumably clotting does not occur when the stream is rapid, because any fibrin-ferment that may be liberated by injured leucocytes or endothelium is swept away before fibrin can become attached to the vessel-wall. Naturally the combination of an injured vessel-wall, a slow current, and a high coagulability offer the most favorable conditions, and we owe to Welch the appreciation of the fact that in a large proportion of all thrombi, even those caused by apparently purely mechanical agencies (*e. g.*, cardiac incompetence), bacteria are present and probably determine the injury to the vessel-walls and the liberation of fibrin-ferment.¹ We have previously referred to L. Loeb's observations on the effect of bacteria in causing coagulation of the blood.

Hyaline thrombi have become of particular interest during the past few years, since it has been shown that they are frequently the cause of extensive degenerative lesions in the viscera, and also because of their relation to the more recently understood *hemagglutinating substances* (see Chap. ix). Although

¹ Welch, Venous Thrombosis in Cardiac Disease, Trans. Assoc. Amer. Phys., 1900, vol. 15.

formed of red corpuscles, these thrombi do not stain at all like normal corpuscles, presumably because a certain proportion of the hemoglobin has been altered or lost through hemolysis. Of particular interest is their reaction to Weigert's fibrin stain, by which they often, but not always, stain intensely; a fact that has been the cause of much confusion in earlier studies. Flexner¹ first appreciated the nature of these thrombi as originating from agglutinated red corpuscles, although Klebs, Ziegler, and others had earlier suggested that hyalin thrombi were formed from red corpuscles. Boxmeyer² independently arrived at the same conclusion as Flexner, in studying hyalin thrombi as the cause of necrosis in the liver of animals infected with the hog-cholera bacillus. Flexner produced hyalin thrombi by injecting corpuscles agglutinated by ricin, or by injecting ricin itself, or hemolytic substances such as ether or foreign serum. As the thrombi become old, the corpuscles lose their form and color and produce the typical hyalin appearance. Pearce³ proved conclusively the dependence of the thrombus formation upon agglutination, for he secured the same results, including the liver necrosis, by injecting specific agglutinating serums. He states that fibrin threads may occasionally be found at the periphery of the larger thrombi, but never in the smaller ones. The tendency of the thrombi to stain like fibrin by Weigert's method is observed particularly when the tissues have been hardened in Zenker's solution. It is extremely probable, from Flexner's observations, that in the thrombosis produced by injecting various toxic substances into the blood, the so-called "*fibrin-ferment thrombosis*," the thrombi are merely agglutinative thrombi, devoid of fibrin; this is undoubtedly true for many of the thrombi observed after poisoning with the powerfully agglutinative snake venoms (see Chap. viii). On the other hand, some, at least, of the hyalin capillary thrombi are undoubtedly composed of soft masses of fibrin which have not become fibrillar, although the successful staining by fibrin stain is not final proof of the fibrinous nature of a thrombus.

Secondary Changes in Thrombi.—The changes that occur in thrombi after they have existed for some time are largely due either to ingrowth of new tissue or to calcification, the latter of which will be considered in a separate chapter. The only other change of interest from the chemical standpoint is the central softening which may occur in any large thrombus,

¹ Jour. Med. Research, 1902 (8), 316.

² Jour. Med. Research, 1903 (9), 146.

³ Jour. Med. Research, 1904 (12), 329; *ibid.*, 1906 (14), 541.

but is particularly often observed in the large globular thrombi found in the heart. The center of the thrombus may be so completely softened that it resembles a sac of pus, the contents, according to Welch, consisting of necrotic fatty leucocytes, albuminous and fatty granules, blood-pigment and altered red corpuscles, and occasionally acicular crystals of fatty acids. Undoubtedly this softening is related to the process of fibrinolysis previously described, and depends upon digestion of the fibrin by leucocytic enzymes; but the fact that the central portion alone undergoes softening is of interest, suggesting that the antibodies for leucocytic proteoses, which Opie¹ found present in normal serum, prevent digestion at the surface of the clot.

EMBOLISM

Emboli offer little of chemical interest, because of the purely mechanical nature of their origin and of the effects they produce. An exception exists in the case of *fat embolism*, for the manner in which the fat is removed from the blood has invited considerable speculation.² Part of the fat is eliminated in the urine, but the problem of how it escapes from the glomerular capillaries is not satisfactorily explained; large emboli undoubtedly lead to rupture of the capillary walls, and probably some fat also escapes through stomata or similar intercellular openings. Fat may also escape in the bile, and some is probably taken up by the tissue and endothelial cells by phagocytosis. Beneke found that the fat becomes partly emulsified by the mechanical action of the blood current, aided to a slight extent by saponification. The larger droplets after lodging in the capillaries are surrounded by leucocytes, to which Beneke ascribes an active part in the removal of the fat as fine droplets by phagocytic action. We may well believe, however, that the lipase of the plasma is an important agent in disintegrating the emboli, although its action is limited because of the relatively small surface which the large drops offer for attack. After fat droplets have been taken into the cells, they presumably are utilized in metabolism like normally acquired fat, as described previously.

Air embolism presents some features of interest from the chemical standpoint, especially in those cases following sudden decrease in atmospheric pressure in persons who have been exposed for some time to pressures considerably higher than

¹ Jour. Exper. Med., 1905 (7), 316.

² Full discussion by Beneke, Ziegler's Beitr., 1897 (22), 343.

that of the atmosphere (diver's palsy, caisson disease, etc.). This form of air embolism is due to the fact that fluids can dissolve much more gas at high pressures than at low pressures; consequently when the abnormally great pressure to which divers, caisson workers, etc., are subjected is too suddenly reduced to that of the atmosphere, the excessive gas that was absorbed during the period of high pressure by the blood and tissue fluids is released, and forms bubbles in the tissues and blood. The bubbles in the nervous tissues may cause paralyses of various sorts, or death; those in the blood may, if in sufficient amount, cause serious or fatal capillary obstruction. The bubbles consist chiefly of nitrogen, because the power of the blood to hold oxygen in combination is so great that not much of this gas becomes freed.¹ Air embolism following obstetrical operations or surgical operations about the neck and chest presents chiefly mechanical features,² and large quantities of air must be present to cause dangerous obstruction to circulation.³ Gas-bubbles may be produced in the blood soon after death by *B. aërogenes capsulatus*, but it is not probable that they are formed before death and cause air embolism.

INFARCTION

The changes that occur in infarcted areas are of much interest in connection with the study of autolysis, for the absorption of the necrotic tissue of aseptic infarcts is purely a matter of autolysis. Jacoby⁴ found by ligating off a portion of a dog's liver, and keeping the dog alive for some time afterward, that in the infarcted tissues so produced leucin and tyrosin could be detected, just as they are found in liver tissue undergoing autolysis outside of the body. So, too, proteoses may appear in the urine when any considerable amount of tissue is cut off from its blood-supply. The processes of autolysis which occur in ordinary sterile infarcts are, however, extremely slow, and it is doubtful if enough of the products are ever in the blood or urine at any one time to be detected or to cause noticeable effects. For example, in an infarct of the kidney which was known to be almost exactly fourteen weeks old, there still remained a layer of necrotic cortex one millimeter thick, quite unabsorbed during this time. If we examine such aseptic

¹ This subject is fully discussed by Leonard Hill in "Recent Advances in Physiology and Biochemistry," London, 1906.

² Review of literature by Wolff, Virchow's Archiv, 1903 (174), 454.

³ See Hare, Amer. Jour. Med. Sciences, 1902 (124), 843.

⁴ Zeit. physiol. Chem., 1900 (30), 149.

infarcts in various stages, we get the impression that the digestion is accomplished by leucocytes acting on the periphery of the infarct, and not entering the dead area deeply, presumably because of a lack of chemotactic substances in the dead cells. On the other hand, it seems probable that the tissue enzymes themselves play an important part in the autolysis, for if we implant into animals pieces of tissue in which the enzymes have been destroyed by heating to boiling, it will be found that the cells and their nuclei remain unaffected for many weeks; whereas if sterile unheated pieces of tissue in which the enzymes are still active are implanted, they lose their nuclear stain and begin to disintegrate relatively soon, without apparent participation by the leucocytes.¹ Ribbert² found that in experimentally produced anemic infarcts in the kidney of rabbits the nuclei retain their staining property well for nearly twenty-four hours, becoming then small and deeply stained, undergoing karyorrhexis, and in large part disappearing from the convoluted tubules inside of forty-eight hours, although some nuclei may persist in the glomerules for three or more days. In human infarcts, Ribbert believes, the process goes on faster, for he has observed here a loss of nuclei within twenty-four hours. These nuclear changes undoubtedly depend upon autolysis, but it is probable that the enzymes concerned reside in the cytoplasm rather than in the nucleus, for I have observed that cells of lymphoid type, with practically no cytoplasm, generally retain their nuclear stain much longer than cells with more cytoplasm; this is particularly noticeable in splenic infarcts, where the Malpighian corpuscles retain their staining affinities much longer than the pulp elements. Whether the destruction of the nuclei is accomplished by the ordinary intracellular proteases, or by special nucleoproteid-splitting enzymes (nuclease,³ etc.), remains to be determined. It is quite possible, however, that the first changes consist of a splitting of the nucleoproteids of the nucleus by the autolytic enzymes, liberating the nucleic acid, which gives the nuclei the characteristic intense staining with basic dyes (*pycnosis*) observed in areas of early anemic necrosis. The nucleic acid may then be further decomposed by the nuclease or similar enzymes. Taken altogether, then, it would seem that the nuclear and cellular alterations that make up the characteristic picture of anemic necrosis are brought about by the intracellular enzymes—an autolytic process. The removal of the dead substance, how-

¹ Wells, Jour. Med. Research, 1906 (15), 149.

² Virchow's Arch., 1899 (155), 201.

³ Sachs, Zeit. physiol. Chem., 1905 (46), 337; Schittenhelm, *ibid.*, 354.

ever, seems to be accomplished rather by the invading leucocytes, through heterolysis. The relatively small part taken by the intracellular enzymes may possibly be due to the seeping through them of alkaline blood-plasma, for autolytic enzymes are not active in an alkaline medium; the leucocytic enzymes, however, act best in an alkaline medium.¹

About the periphery of infarcts is usually observed more or less fat formation (Fischler²), particularly in the endothelial cells (Ribbert). This is not peculiar to infarcts, however, for Sata³ found a similar peripheral fatty metamorphosis common to all necrotic areas. The basis of this is probably the persistence of the cell lipase, which acts upon fatty acid and glycerin diffusing into the necrotic area with the plasma, unchecked by the normal oxidative destruction of these substances. (See "Fatty Degeneration," Chap. xiv.)

Hemorrhagic infarcts offer, in addition to the changes common to anemic infarcts, the alterations occurring in the blood-corpuscles. Panski⁴ found that after ligation of the splenic vein of dogs the red corpuscles begin to give up their hemoglobin in about three hours. After twelve hours fibrin formation begins in the tissues, the corpuscles continue to give up hemoglobin and become cloudy in appearance. Later, iron-containing pigment is formed in the cells beneath the capsule, but in the deeper tissue even the iron normally present in the spleen tissue seems to disappear; this probably depends upon the fact that pigment reacting for iron, hemosiderin, is formed only in living cells under the influence of oxygen. The hemolysis is probably produced either by the action of autolytic products, which are notoriously hemolytic, or perhaps also by direct attack of tissue and blood proteases upon the corpuscles.

Other retrogressive changes that may occur in infarcts, such as septic softening and calcification, are not greatly different from the same processes occurring in other conditions, and will be considered with the discussion of these processes.

¹ More fully discussed by Wells, *loc. cit.*

² *Cent. f. Path.*, 1902 (13), 417.

³ *Ziegler's Beitr.*, 1900 (28), 461.

⁴ "Untersuchungen über den Pigmentgehalt der Stauungsmilz," Dorpat, 1890.

CHAPTER XII

EDEMA ¹

As the term edema indicates the excessive accumulation of lymph (which may be either normal or modified in composition) in the cells, intracellular spaces, or serous cavities of the body, the problems of edema are inseparably connected with the consideration of the processes of physiological formation and removal of lymph. For many years the study of these processes has been a favorite field of investigation by physiologists, and the great battle-place of the "vitalism" and "mechanism" schools; and to this day the forces that determine the formation of lymph and its subsequent absorption have not been completely understood. By the application of the principles of physical chemistry to the problem, however, great advances have recently been made, which seem to render our understanding of both lymph-formation and its pathological accumulation in the tissues much clearer and more nearly accurate than they were before. We shall first consider, therefore, the physiological formation of lymph, before taking up the subject of edema.

Composition of Lymph.—Lymph consists of material derived from two chief sources. The greater part consists of fluid passing out of the capillaries into the tissue-spaces; here it is modified by the addition of products of metabolism derived from the tissue-cells, and by the subtraction of materials that the cells utilize in their metabolism. It is, therefore, essentially a modified blood plasma, and the modifications the plasma undergoes are so slight that, under ordinary conditions, lymph shows on analysis no considerable differences from blood plasma, except a relative poverty in proteids, due chiefly to the impermeability of the capillary walls for colloids. Its quantitative composition varies greatly, depending upon the conditions under which it is collected, whether during activity or rest, etc. The following tables of analyses have been collected by Hammarsten:²

¹ A complete bibliography is given by Meltzer, *American Medicine*, 1904 (8), 19 *et seq.*, and references will be given below only when referring to special points or to articles not included by Meltzer. Literature also reviewed by Burton-Opitz, *Jour. Amer. Med. Assoc.*, 1899 (32), 51, and by Ellinger, *Ergebnisse der Physiol.*, 1902 (I, Abt. 1), 355.

² *Physiological Chemistry*; Amer. translation, 1904, p. 213.

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0.4	2.2
Albumin	42.7	42.8	34.7	35.0
Fat, cholesterin, lecithin . .	3.8	9.2	. .	
Extractive bodies	5.7	4.4	. .	
Salts	7.3	8.2	7.2	7.5

1 and 2 are analyses of lymph from the thigh of a woman, 3 is from the contents of sac-like dilated vessels of the spermatic cord, 4 is lymph from the neck of a colt.

Chyle differs from lymph chiefly in the presence of large quantities of fat; during starvation the lymph and the chyle are of practically the same composition.

Normal lymph contains much less fibrinogen than does the blood plasma, and hence coagulates slowly. Lipase and other enzymes have been found in the lymph, as in the plasma. The products of tissue metabolism added to the lymph by the cells may render it toxic (Asher and Barbera¹). Under pathological conditions the lymph may be greatly altered, becoming poorer in solids under some conditions of edema, and becoming rich in proteids and blood-corpuscles under inflammatory conditions, until it partakes of the characteristics of an inflammatory exudate (see analyses of transudates and exudates).

FORMATION OF LYMPH²

Filtration Theory.—The simplest possible conception of lymph formation is that it is simply the result of filtration of the liquid constituents of the blood through the capillary walls under the influence of the blood pressure. This “filtration theory” was supported originally by Ludwig, and it was a prominent factor in the early applications of mechanical principles to biological processes. In support of this theory were advanced the results of numerous experiments in which it was shown that increasing the blood pressure by means of ligating the veins, or by causing arterial dilatation, resulted in an increase of the lymph flowing out of the lymph-vessels of the part. The experimental results were not always favorable to the theory, however, particularly in the experiments in which blood pressure was raised by arterial dilatation; often the flow of lymph was little increased, even when the arterial flow and pressure were greatly increased. Furthermore, as Leonard Hill³ has urged, there is reason for questioning the existence of such a thing as a “filtration pressure” in organs or tissues provided with a capsule, since within this capsule all structures must be

¹ Zeit. f. Biol., 1898 (36), 154.

² See review by Asher, Biochem. Centralblatt, 1905 (4), 1.

³ Biochemical Journal, 1906 (1), 55.

under equal pressure, which is the pressure of the blood ; therefore there is the same pressure both on the inside and on the outside of the capillary walls. Nevertheless, the filtration theory held for many years, not only as an explanation of lymph formation, but also as an explanation of urinary secretion and of the secretion by other organs. It was only within a comparatively short time that it became clear that filtration alone could not account for all the phenomena of secretion. For example, in many lower forms with undeveloped circulatory systems, and almost no blood pressure, secretion goes on vigorously ; the pressure of glandular secretions may be much higher than the blood pressure within the capillaries ; the activity of secretion is by no means in proportion to blood pressure, etc. If in glandular secretion, therefore, fluids are removed from the blood and transferred into an excretory duct through the action of some force other than that of the blood pressure, it is probable that lymph formation is equally independent of blood pressure. On this basis Heidenhain advanced his—

Secretory theory of lymph formation, in which he suggested that lymph is the product of an active secretion by the endothelial cells of the capillaries, just as saliva is the product of the activity of the glandular cells. He showed that certain chemical substances may stimulate lymph flow, independent of blood pressure, just as pilocarpine and other drugs may stimulate the secretion of saliva. These lymph-stimulating substances, which he named *lymphagogues*, fall into two distinct classes. One, which includes such substances as peptone, leech extract, strawberry juice, extracts of crayfish, mussel or oysters, and numerous other tissue extracts, are characterized by causing the secretion of a lymph which is rich in proteids, even richer in proteids than the blood plasma ; and, furthermore, there is no simultaneous increase in urinary secretion. Heidenhain considered that these substances caused lymph secretion by stimulating the capillary endothelium in a specific manner ; as they caused no appreciable rise in blood pressure the increased lymph secretion certainly could not be attributed to filtration. This independence of the lymph flow on blood pressure is most conclusively shown by *postmortem lymph secretion* ; for example, Mendel and Hooker¹ observed lymph flow for four hours after death, in a dog that had received an injection of peptone eight minutes before being killed.

The *second class* of lymphagogues includes crystalloidal substances, such as sugar, urea, and salts. Lymph secreted under

¹ Amer. Jour. of Physiol., 1902 (7), 380.

the influence of these substances is poorer in proteids than ordinary lymph, and at the same time an increased urinary secretion is produced. With these crystalloidal lymphagogues the amount of effect is in inverse proportion to their molecular weight, which means that their effects depend upon the number of molecules in solution rather than upon their nature; in other words, the stimulation of lymph by crystalloids is dependent upon the osmotic pressure of the crystalloids. Heidenhain explained their action as follows: The crystalloids are secreted into the lymph-spaces by the action of the capillary endothelium, and there, owing to their raising osmotic pressure, cause a flowing of water out of the vessels. The difficulty here is to explain why the crystalloids while still in the vessels do not attract the fluids from the lymph-spaces into the blood, and so cause rather a *lessened* lymph secretion.

While admitting that in pathological conditions (*e. g.*, passive congestion) pressure and filtration *may* play an important part, Heidenhain considered that an active secretion by the endothelial cells is the chief factor in the normal formation of lymph. The means by which the cells perform this function was unknown; it was considered as an example of "vital activity," Heidenhain meaning by this term such chemical and physical forces of living cells as are unknown or not understood at the present time, rather than any metaphysical conception of living matter, such as many vitalists assume.

Other observers, corroborating Heidenhain's results for the most part, have modified or amplified his theory. Asher and his collaborators, for example, ascribe the work done in causing lymph formation to the cells of the various tissues and organs, rather than to those of the capillary wall. The increased flow of lymph from the salivary gland that occurs during its activity they consider due to the work of the gland cells, and its function the removal of products of metabolism. The action of such a lymphagogue as peptone they ascribe to its stimulation of cellular activity, particularly in the liver, where it causes an increased formation of bile. Gies¹ and Asher also observed that after injection of crystalloidal lymphagogues, such as sugar, a prolonged flow of lymph occurred after the death of the animal, proving completely that such lymphagogic action is independent of blood pressure.

Potocytosis.—In explanation of the process by which the cells, whether endothelial or tissue-cells, pass fluids through themselves from

¹ Amer. Jour. Physiol., 1900 (3), p. xix; Zeit. f. Biol., 1900 (40), 207.

one place to another, Meltzer¹ has made an interesting suggestion, as follows: Considering the property of endothelial cells to act as phagocytes, MacCallum² has shown that solid granules (*e. g.*, coal pigment, carmin) are taken through the walls of the lymphatics by the phagocytic activity of their endothelial cells. Meltzer suggests that in a similar way the endothelial cells may transport through the vessel-walls not only solid particles, but also, by the same mechanism, substances in solution; and for this hypothetical process he suggests the name "*potocytosis*." There can be little question that cells do take up substances in solution, and sometimes this is done in an apparently selective manner; *e. g.*, the taking up of bacterial toxins and vegetable poisons in the peritoneal cavity by the leucocytes. Presumably the mechanism of "*potocytosis*" is not different from that of phagocytosis, chemotactic forces determining the occurrence of the process. No experimental evidence has been advanced as yet for this very plausible hypothesis.

Permeability of Capillaries.—In explanation of the variability in the amount and composition of the lymph, Starling³ has introduced the factor of altered permeability of the capillary walls, which presumably depends upon the number and size of the pores. He found that normally the lymph coming from the lower extremities contains only 2 per cent. to 3 per cent. of proteids, while lymph from the intestines contains 4 per cent. to 6 per cent., and lymph from the liver contains 6 per cent. to 8 per cent. of proteids; hence he considers that the liver capillaries are the most permeable, *i. e.*, have the largest pores, so that more of the large colloid molecules can escape from them. The effect of lymphagogues of the first class (peptones, etc.) he attributes to their poisonous properties, and the consequent injury to, and alterations in, the capillary wall. The crystalloidal lymphagogues, he believes, act by first attracting fluids from the tissues into the blood with a resulting "hydremic plethora," which in turn leads to increased blood pressure and consequent filtration of a watery fluid out of the vessels. He considers, therefore, that the amount and quality of the lymph produced in any part are determined solely by two factors, the intracapillary blood pressure and the permeability of the capillary walls.

In connection with this question of the permeability of the capillary walls, Meltzer suggests that the contractility and irritability of the endothelium may be a potent factor in determining the size of the pores in the capillary walls. When in a tonic condition, the endothelium is firmly contracted about the pores, keeping their size small; when the endothelial cells

¹ *Loc. cit.*

² Johns Hopkins Hosp. Bull., 1903 (14), 1.

³ Lancet, 1896 (i), May 9, *et seq.*; Schäfer's Text-book of Physiology, vol. 1.

become relaxed by any cause, such as poisons, high blood pressure, poor nourishment, etc., the pores are enlarged, and increased escape of fluids results. It must be borne in mind, however, that not all histologists admit that capillary walls contain pores.

Osmotic Pressure.—Still another important factor in causing fluid to leave the vessels is osmotic pressure. Heidenhain refers to this cause the transudation produced by crystalloid lymphagogues, although in a rather unsatisfactory manner. As a result of the more recent studies of physical chemistry, and its application to biological processes, we have learned to appreciate the importance of osmotic pressure in cell activities (see Introductory Chapter), and in the question of lymph formation it occupies a particularly important place. We may consider it as follows: In the blood we have certain proportions of readily diffusible crystalloids and of non-diffusible colloids. If no metabolic processes were going on in the tissues, we should have the diffusible substances leaving the vessel-walls (leaving out, for the present, any question of activity on the part of the endothelium) until an osmotic equilibrium is established in the tissues and in the blood. As a matter of fact, however, the blood proteids are not absolutely non-diffusible, but small quantities do pass through the capillary walls, and so lymph under such a hypothetical condition would consist of a mixture of the same osmotic concentration as the blood plasma, with about the same proportion of crystalloids, but a smaller proportion of proteids; this, it will be noticed, is just about the composition of normal lymph. During life, however, the cells of the tissues are causing metabolic changes in these lymphatic constituents, and these changes consist chiefly in breaking down large molecules of proteids, carbohydrates, and fats into much smaller molecules. Now the osmotic pressure of a solution is dependent upon the *number* of molecules and ions it contains, hence by breaking down these few large molecules with very little osmotic pressure into many small molecules, the osmotic pressure in these cells and tissues becomes raised above that of the blood-vessels, and consequently water flows out of the vessels because of the increased pressure. We see here the probable explanation of the stimulating influence of metabolic products upon the formation of lymph, noted by Hamburger, Heidenhain, and others. For suggesting and urging the importance of osmotic pressure in the formation of lymph we are indebted particularly to Heidenhain, v. Korányi,¹ J.

¹ Zeit. f. klin. Med., 1897 (33), 1; 1898 (34), 1.

Loeb,¹ and Roth.² Loeb shows very clearly the relative greatness of the water-driving force of osmotic pressure as compared to that of blood pressure, by his statement that the osmotic pressure of a physiological salt solution is about 4.9 atmospheres, which is *twenty times as great as the blood pressure* with which we have to do in ordinary physiological experiments. In varying osmotic conditions we may readily see an explanation for the increased lymph flow that occurs during tissue activity; namely, it is due to the increased formation of metabolic products. Many of the lymphagogues may act similarly by stimulating metabolic activity, with resulting increase in the formation of osmotic pressure-raising products of metabolism in the organs; *e. g.*, the increased lymph flow from the thoracic duct that follows stimulation of hepatic activity by injection of peptone (Heidenhain) or ammonium tartrate (Asher and Busch³). As we shall see later in considering edema, osmotic pressure plays an important part in the pathological formation of lymph.

Summary.—We see from the above discussion that numerous theories have been advanced to explain the normal formation of lymph, and as their basis exist several different possible factors. Filtration, active secretion by the capillary endothelium, attraction by the tissue-cells, osmosis in response to formation of crystalloids outside the vessels; all have been shown to be possible causes of lymph formation. It is highly probable that in a certain way all are involved, particularly if we accept the view of the physical school that “secretion” and “attraction” by the cells are merely the outcome of osmotic forces; the causes of lymph formation then reduce themselves to two, filtration and diffusion. There has been, until recently, no question but that lymph does escape from the vessels through simple filtration, for the pressure inside the capillaries is presumably greater than outside, the capillary walls are not watertight, and they are not impermeable to the substances dissolved in the plasma.⁴ Likewise osmotic exchanges surely go on between the vessels and the tissue-cells. The question that remains is, do these two factors account for all of the lymph formation, and are they sufficient by themselves to explain the

¹ Pflüger's Arch., 1898 (71), 457.

² Englemann's Arch., 1899, p. 416.

³ Zeit. f. Biol., 1900 (40), 333.

⁴ Hill (“Recent Advances in Physiology and Biochemistry,” 1906, p. 618) disputes the possibility of such a thing as filtration pressure, on the ground that the structures within the capsule of an organ must all be alike under the influence of the blood pressure.

physiological regulation and the pathological variations in the lymph flow? They are purely physical or mechanical causes, and the "vitalist" school will claim that they are inadequate and that "vital activities" of the cells play the deciding rôle. But at present the evidence that is being accumulated seems to point more and more strongly to the conclusion that these "vital activities" are but the result of simple well-known physical forces acting under very complex conditions—complex because of the large number of very different chemical compounds occurring together, and the varying influence of circulation, food supplies, cell structure, etc.

ABSORPTION OF LYMPH

By no means all the fluid that escapes from the vessels, nor all the products of cell metabolism are carried away in the lymph—a considerable and perhaps the greater part of them is absorbed back into the capillaries directly. A classical proof of this is the experiment of Magendie, who observed that if poisons were injected into the leg of an animal, which had been separated from the body entirely except for the blood-vessels, that poisoning developed in the usual manner. In such experiments the lymph-vessels are severed and probably largely occluded, hence it does not solve the question as to whether substances are absorbed by the blood-vessels under normal conditions. Orlow found, however, that during absorption of fluid from the peritoneal cavity there is no perceptible increase in the lymph flow from the thoracic duct. Addition of sodium fluoride, a protoplasmic poison, was found to interfere with this absorption, for which and other reasons Heidenhain and Orlow considered that the absorption depended upon the "vital activity" of the cells. More nearly reproducing normal conditions were the experiments of Starling and Tubby, who found that methylene-blue or indigo-carmin injected into the pleura or peritoneum appeared in the urine long before it colored the lymph in the thoracic duct.¹ Adler and Meltzer found evidence, however, that not all the absorption is accomplished by the blood-vessels, for obstruction of the thoracic duct retards absorption. That the absorption is not dependent solely upon the circulation and blood pressure is shown by the fact that absorption from the peritoneal cavity occurs in dead bodies (Hamburger, Adler and Meltzer).

The nature of the mechanism by which fluids are taken into

¹ See Mendel, *Amer. Jour. Physiol.*, 1899 (2), 342.

the blood-vessels is still unknown. We can easily understand the entrance of injected poisons and coloring-matters from the tissues into the blood, because they are more concentrated at the point of injection than in the blood, hence they may diffuse directly through the capillary wall. Likewise we can understand the diffusion of water from a hypotonic solution into the blood, but how a solution of the same concentration as that of the blood can enter the blood is difficult to explain. Cohnstein and also Starling attribute this absorption to the proteids of the blood in the following manner: After a fluid is injected into the tissues or serous cavities there occurs a diffusion exchange between this fluid and the blood, until the concentration of the crystalloids in each is equal; but the proteids of the blood cannot diffuse, and as they exert a positive although very slight osmotic pressure, this difference in osmotic pressure in favor of the blood causes diffusion of the extravascular fluid into the blood. Roth has also applied this idea in a rather complicated manner to the absorption occurring in metabolic processes (see Meltzer), but it must be admitted that it is an unsatisfactory solution of the problem.

Passage of the fluid from the tissues into the lymph stream was very easy to understand in the light of the older conception of the lymphatic circulation, namely, that the lymph-vessels were merely continuations of the interstitial spaces; we could then assume that as soon as the fluid left the blood-vessels it was practically within the lymphatic system, and was crowded along the lymphatic channels by the *vis a tergo*, aided by the valves of the lymph-vessels and the intrathoracic vacuum. But it now seems, particularly through the studies of MacCallum,¹ that the lymphatic vessels form a closed system, not in communication with the interstitial spaces. This being the case, we have to explain the passage of the lymph through the walls of the lymphatic vessels, and this is a problem which is not by any means a simple one, and which has yet to be investigated.

THE CAUSES OF EDEMA

With the facts and hypotheses mentioned in the preceding paragraphs in mind, we may consider their bearing on the production of abnormally large accumulations of lymph in the tissues, that is, edema. We can imagine any one of the following factors as causing or helping to cause such a pathological accumulation:

¹ Johns Hopkins Hosp. Bull., 1903 (14), 1.

1. Obstruction to outflow through the lymph-vessels.
2. Increased blood pressure.
3. Decreased extravascular pressure.
4. Increased permeability of the capillary walls.
5. Increased filterability of the blood plasma.
6. Osmotic pressure changes—either an extravascular increase or an intravascular decrease.

These may be taken up one by one, and considered in relation to their bearing upon the general problem of edema.

1. Obstruction to Outflow through the Lymph-vessels.—Because of the very abundant anastomosis of the lymphatic vessels it is extremely difficult or impossible to cause any appreciable obstruction to the lymphatic circulation by ligation of lymphatic trunks in the limbs or organs of the body, and in pathological conditions this possible cause of edema is seldom actually observed. The chief instance of edema from lymphatic obstruction is observed after occlusion of the thoracic duct by tumors, tuberculous processes, animal parasites, or thrombosis; such occlusion is usually followed by rupture of the duct or its tributaries, with the production of *chylous ascites* or *chylothorax*, and *chyluria*. *Filaria* or their ova may occupy so many of the lymphatic channels of an extremity (leg) or part (scrotum) that the anastomotic channels are thoroughly blocked, with a resulting local edema that in course of time is followed by the production of inflammatory connective tissue and elephantiasis.¹ Chronic lymphangitis may also result in lymphatic obstruction to such an extent that chronic edema results.

Another way in which edema may be caused or influenced by lymphatic obstruction is generally overlooked, but it is possibly of great importance; namely, from *pressure upon the lymph channels* by dilated vessels in hyperemia, or by cellular exudates and swollen tissues in inflammation. We see evidence of this in the rapid absorption of exudates that frequently follows the removal of but a part of the fluid in a chest cavity; apparently the decrease in pressure frees the paths of absorption and permits them to take up the remaining fluid. In inflammatory edema the lymphatic obstruction is probably not great, for Lassar found that the amount of lymph escaping from an edematous extremity is much greater than from a normal one; but in the case of strangulated hernias or other conditions in which edema results from circular constriction, obstruction of the lymphatic vessels may be a factor of no mean importance.

There is no difficulty in understanding edema from the above

¹ Manson, Allbutt's System, 1897 (ii), 1082.

causes—it is simply a passive congestion of the lymphatic circulation, and no chemical factors are involved. The nature of the fluid found in such forms of edema will be discussed later.

2. Increased Blood Pressure.—This takes us back to the filtration theory of lymph formation, and as it is generally conceded that more or less fluid escapes from the vessels by this mechanical process, the questions to be decided are: Can and does increased blood pressure, alone and without other aiding factors, cause edema? If not, does it play an auxiliary part in producing edema, and how important a part may this be? Many experiments have been performed with the object of answering these questions, with more or less conflicting results. Cohnheim demonstrated that vasodilation (active hyperemia) alone will never bring on an edema; and many observers state that ligation of the femoral or other large veins will not cause edema in animals. However, when the vein is occluded, and the arteries are dilated by cutting their vasoconstrictor nerves, then edema may result (Ranvier, Cohnheim); but whenever venous outflow is impeded, we have other factors than simply increased pressure to consider, for the nourishment of the parts is decidedly impaired, and, as we shall see later, this may be of much greater importance than is the associated rise in blood pressure. To produce edema in the lungs by mechanical forces it is necessary to ligate the aorta and its branches, or the pulmonary veins (Welch). As such high pressures do not occur in any pathological conditions, it is safe to assume that increased pressure alone is not capable of causing by itself the pulmonary edema so frequently observed clinically. Welch,¹ however, has supported the hypothesis that a disproportion between the working power of the left ventricle and of the right ventricle may lead to pulmonary edema through pulmonary hyperemia. In the edema of passive congestion generally, increased blood pressure would seem to be an important factor, and there is no doubt that with an increased pressure of the degree observed in such conditions some increase in the lymph flow would result; but from the evidence at hand it is improbable that the amount of lymph so secreted would ever be more than the lymph-vessels could carry away. Even the added obstruction to lymphatic flow due to pressure upon the lymph capillaries by congested blood-vessels, and the resistance to the lymph escaping from the thoracic duct offered by the increased pressure in the subclavian vein, would not satisfactorily account for the edema of cardiac incompetence. Not to go into details here, it may be stated that

¹ Virchow's Arch., 1878 (72), 375; see also Meltzer (*loc. cit.*).

the impression is growing that uncomplicated rise in blood pressure is not sufficient by itself to produce edema. Some of the reasons for belittling this factor will be brought out in the subsequent discussion.

3. Decreased Extravascular Pressure.—This factor is particularly prominent in the so-called "*edema ex vacuo*," which occurs after the absorption of an area of tissue which is so located that the surrounding tissues cannot contract or fall in to fill the gap, *e. g.*, brain softening, serous atrophy of fat. A still better example, however, is the edema that follows local decrease in atmospheric pressure in "cupping." In these instances the edema depends partly upon increased transudation, and partly on the retention of the fluid in the tissues, because it cannot well leave them against the atmospheric pressure. The idea advanced by Landerer that decreased elasticity of the tissues was a possible cause of edema has been disproved by Bönniger,¹ who found but little alteration in the elasticity of tissues the seat of edema. *Edema ex vacuo* is again an illustration of edema due to purely mechanical causes, but it is of little practical importance.

4. Increased Permeability of the Capillary Walls.—The importance of this factor in the production of edema was first demonstrated by Cohnheim and Lichtheim, who found that the production of an enormous increase in the amount of fluid in the blood (hydremic plethora) by injecting large quantities of salt solution, caused an edema of the viscera and serous cavities, but not any subcutaneous edema until the skin had been irritated by some means, such as hot water, iodine, etc. By this irritation the capillary walls are injured, and an excessive escape of the blood fluids follows. Magnus also showed that poisoning with arsenic, which injures the vessels, favored the experimental production of edema by transfusion. Starling, as noted before, observed that the permeability of the capillaries varies normally in different organs and tissues, which determines quantitative and qualitative differences in the lymph normally flowing from various vascular areas. Heidenhain's "lymphagogues of the first class," which are all poisonous substances, probably act by increasing the permeability of the capillaries, and in this way they produce *local urticaria*, which is often observed as a result of poisoning by these same lymphagogues, *e. g.*, shellfish and strawberry poisoning. Just what changes are produced in the capillary walls that renders them more permeable we do not know. Possibly in some instances it is a

¹ Zeit. exp. Path. u. Ther., 1905 (1), 163.

partial solution of the intracellular cement substances, possibly an enlargement of the stomata through loss of tonicity of the endothelium (Meltzer), sometimes it may be actual death of the endothelial cells, or, as Heidenhain and Cohnheim thought, it may be a stimulation of the endothelial cells to increased secretory activity.

Under pathological conditions increased permeability of the capillary walls is probably one of the chief factors in the production of certain forms of edema. We see evidence of it particularly in inflammatory edema, with its proteid-rich exudate. It cannot be doubted that in such conditions actual physical alterations take place in the capillaries, when we see that the slightly diffusible proteids escape from the vessels in the same proportions as they exist in the plasma; there can be here no question of heightened cell activity or increase in osmotic pressure, especially not when we note the indistinguishable transition of such an inflammatory exudate into one containing leucocytes and red corpuscles, which must pass through openings of some kind in the vessels. Edema due to inflammation and poisoning certainly depends to a large degree upon alterations in the vessel-walls. The question remaining is, do edemas that are not associated with distinct inflammatory or toxic influences depend also upon the vascular permeability?—does increased permeability ever lead to the formation of proteid-poor transudates? Cohnheim was inclined to attribute nearly all edema to this cause, for in passive congestion, or nephritis, or any of the common causes of edema, it is easy to find reason for the belief that poisons may be present in the blood; and as there was good evidence that the blood pressure alone could not account for the edema, it was natural to ascribe all these forms of edema to the action of toxic substances upon the capillary walls, leading to increased permeability; or, what might amount to the same thing, increased secretory activity of the endothelium, as understood by Heidenhain. It is impossible at this time to eliminate as non-existent this secretory-activity doctrine, but, as we hope to show later, there exist other factors in all these non-inflammatory edemas that are sufficient to account for the edema without our having recourse to this hypothesis. For the present, therefore, we may consider altered capillary permeability as an essential factor in edemas characterized by proteid-rich fluids (exudates), and state that the influence of altered permeability in the production of proteid-poor fluids (transudates) is not proved, and is perhaps not of importance.

5. Increased Filterability of the Blood Plasma.—

This takes us back to Richard Bright's conception of renal dropsy. He imagined that through the great loss of albumin in the urine the blood became so thinned and watery that it could filter through the vessel-walls, while normal plasma, he thought, was too thick and viscid to do so. The same idea was applied to the edemas of cachexia in cancer, etc., chlorosis, and all forms of edema associated with a decrease in the corpuscular or proteid elements of the blood. With our present knowledge of diffusion of crystalloids and colloids we can readily appreciate that a decrease in the blood colloids, such as might occur in these diseases, could not modify the passage of fluids through the capillary walls to any considerable degree. Stewart and Bartels considered that in renal dropsy the increased filterability of the plasma was not due so much to the loss in albumin as to retention of water, which caused an hydremic plethora. But this factor was soon eliminated, for it was found that complete anuria, produced by ligating both ureters, does not cause edema; and also that to produce an edema by increasing the water of the blood it was necessary to increase it many times as much as it can ever be increased by disease. Simply increasing the proportion of water by removing part of the blood and injecting a corresponding amount of salt solution did not cause edema (Cohnheim and Lichtheim). We may, therefore, look upon the hypothesis of increased filterability of the blood as chiefly of historic interest, and not an important factor in the causation of edema.

6. Disparity of Osmotic Pressure in Favor of the Tissues and Lymph over the Blood.—

On a preceding page we have already considered the means by which changes in osmotic pressure in the tissues are brought about, and how they may lead to an accumulation of fluid. The importance of osmotic pressure in causing pathological edema was suggested by Loeb¹ in his studies on the physiological action of ions. He stated that edema occurred when the osmotic pressure was higher in the tissues than it was in the blood and lymph, and the cause was to be sought in conditions that lowered the osmotic pressure of the blood and lymph or raised that of the tissues. This condition he found in the accumulation of metabolic products:—in the case of muscle, tetanization of a frog's muscle for ten minutes raised the osmotic pressure over one atmosphere; separating a muscle from its blood-supply led to such an increase in osmotic pressure that it took up water from a 4.9 per cent.

¹ Pflüger's Arch., 1898 (71), 457.

NaCl solution, which has a pressure of *over thirty atmospheres*. When we consider that in his studies on lung edema Welch was able by ligation of the aorta to raise the blood pressure less than $\frac{1}{10}$ atmosphere, we begin to appreciate how much more powerful are the forces of osmotic pressure that are at work in the body than is the blood pressure, even of the aorta itself.

Loeb found that whenever oxidation is impaired in a tissue its osmotic pressure rises, due to the accumulation of incompletely oxidized metabolic products, particularly acids, and as a result the muscle takes up water and becomes edematous. On this basis we may explain the edema of venous stagnation as due to accumulation of products of metabolism, partly because of impaired oxidation, partly, perhaps, because of their slow removal in the blood on account of the circulatory disturbance. The so-called "neurotic" edemas may possibly be explained by local increase in metabolic activity brought about by nervous stimuli, which causes increased formation of substances raising osmotic pressure in the stimulated tissues. In renal edema the retention of water also seems to depend rather on osmotic pressure than on circulatory disturbances or alterations in the vessel-walls, for it has been shown that retention of chlorides, which the diseased kidneys do not eliminate normally, is an important cause of the dropsy. The chlorides accumulating in the tissues lead to an increased osmotic pressure, which causes the abstraction of water from the blood and its retention in the tissues. (The details of this subject will be considered later.) Conversely, Meltzer and Salant found that salt solution is absorbed from the peritoneal cavity more rapidly in nephrectomized rabbits than in normal rabbits, because metabolic products accumulate in the blood and raise its osmotic pressure above normal.

There are some difficulties, however, in applying the influence of osmotic pressure as an explanation of all edemas. For example, in edema of the lungs, as Meltzer points out, what is the force that drives the fluid into the empty air-cells? Equally difficult to explain as the result of osmotic disturbance is the distribution of fluid that is seen in cardiac dropsy. The fluid does not accumulate in the tissues where metabolism is greatest, or where the most oxygen is used; but rather in the inactive subcutaneous tissues and in the serous cavities. Possibly the original transudation does occur in the muscles and solid viscera, and the fluid is then mechanically forced out of them into the surrounding tissue-spaces, later settling according to the laws of gravity or according to the distensibility of the tissues.

Summary.—We find that a number of factors may be considered as responsible for edema, some of them being prominent in one instance, some in another, but *in few cases can we consider one factor alone as the sole cause.* In most of the forms of edema, such as those due to renal disease and cardiac disease, it now seems probable that osmotic pressure changes play the most important part; whereas in inflammatory edema there can be no question that alteration in the capillary walls is the most essential factor. But the mechanical factor of blood pressure cannot be disregarded, although by itself seldom sufficient to cause edema; associated with other factors it is undoubtedly an important agency, for there are few edemas that are not associated with increased blood pressure. Hydremia and hydremic plethora may almost be disregarded, except in so far as they may cause altered metabolism in the tissues, injury to vessel-walls, and decreased osmotic pressure within the vessels. Lymphatic obstruction is possibly a factor of some secondary importance if we consider that distended vessels and tense tissues may occlude the lymph capillaries.

SPECIAL CAUSES OF EDEMA

We may now consider which of the above factors are at work in bringing about edema under the conditions in which it is usually observed clinically.

“Cardiac” Edema.—Passive congestion introduces nearly all these factors, for in addition to the increased blood pressure there is also an opportunity for changes in the capillary wall, either from stretching and thinning of the cells and cement substances, or from “loss of tone” in the endothelium surrounding the stomata (Meltzer), or from toxic injury by accumulated products of tissue metabolism. When the stasis is nearly complete, or if it is complete for a time and then relieved, the endothelium may be injured through lack of nourishment. As the edematous fluid in passive congestion is usually of a watery type, poor in proteids, the edema is probably less dependent upon capillary permeability than upon other factors, except in the case of acute stasis, when the fluid partakes of the character of the exudates. Undoubtedly the accumulation of crystalloids within the tissues also plays a most important part in this form of edema, Loeb’s experiments having shown how greatly osmotic pressure is raised in tissues having deficient oxygen supply. Finally, there is probably more or less obstruction to lymphatic outflow because of the increased pressure upon the lymphatic

channels, and perhaps also, in the case of cardiac incompetence, obstruction to the discharge of lymph from the thoracic duct into the subclavian vein against the high intravenous pressure.

Renal Edema.—We must recognize under this heading two different types of edema. In acute nephritis (*e. g.*, in scarlatina) toxic materials appear to be the chief cause, and, as Senator contends, injure alike the capillaries of the renal glomerules and of the subcutaneous tissues; in each case there results an increased permeability which is manifested by albuminuria as a result of the injury to the renal capillaries, and by edema as a result of the injury to the tissue capillaries. This sort of edema is allied to that produced by peptone and similar lymphagogues, and we might well imagine that the mechanism consisted merely in an injury to the capillaries through which excessive fluid is driven by the blood pressure, were it not for such observations as those of Mendel and Hooker,¹ who found that postmortem flow is increased by these lymphagogues also. We can hardly account for the force exhibited in postmortem lymph flow on any other ground than that it is furnished by osmotic pressure, unless we wish to fall back upon "vital activity" of the surviving cells. Hence it is probable that even in the edemas of toxic conditions, such as acute nephritis, osmotic pressure plays a part, the pressure-raising substances probably being abnormal or excessive metabolic products of the cells affected by the poisons.

In the more common edema of chronic nephritis we have to consider, among other factors, the blood pressure. That this is not an essential or even important cause, however, is shown by the fact that edema is usually much less marked in interstitial nephritis with high blood pressure than it is in parenchymatous nephritis with a much lower pressure. Toxic substances are, of course, also present in the blood, and may alter capillary permeability; these toxic substances may account for the localized edemas and erythemas sometimes observed in nephritis. But *probably most important is the action of the crystalloids* which the kidney does not excrete, and which seem to be stored up in the tissues, where they cause transudation of water under the influence of their osmotic pressure. For example, Rzentkowski² found that the average lowering of the freezing-point by the edematous fluid in nephritis was 0.583° , in cardiac dropsy it was 0.548° , and in tuberculous pleuritis 0.526° . This indicates that the osmotic concentration of the fluid is highest in renal

¹ Amer. Jour. of Physiol., 1902 (7), 380.

² Berl. klin. Woch., 1904 (41), 227.

dropsy, and supports the belief that here and in cardiac dropsy osmotic pressure plays a more important part than it does in inflammatory exudation. Of the crystalloids that cause accumulation of fluid in the tissues, sodium chloride seems to be the most important.

Retention of Chlorides in Edema.¹—From the investigations made by numerous clinicians, especially the French, there seems to be no question but that—(1) in nephritis with edema a retention of sodium chloride frequently occurs; (2) that elimination of the chlorides is often increased during periods of improvement of the edema; (3) that a reduction of the amount of chlorides in the diet often causes a great improvement in the edema, while administration of chlorides may make the edema much worse. There are, however, observations that also indicate that chloride retention does not account for all cases of renal dropsy, for many instances have been observed in which the above-mentioned conditions were not fulfilled. Nevertheless, it cannot be denied that chloride retention is often an important causative factor in the edema of parenchymatous nephritis. If the retained chlorides obeyed the ordinary laws of diffusion, we should expect them to become distributed alike in the blood and tissues, so that they would merely cause an equal increase in the fluids of the blood and of the tissues; that is to say, there would be an hydremic plethora due to retention of water in the body by the accumulating chlorides. But, according to a number of observers, there is a specific retention in the tissues, which Strauss calls "*historetention*," and which explains the local edema. The way in which the historetention is produced is, however, not understood, and not all observers accept this hypothesis (Scheel²). In many conditions other than nephritis, there is also a chloride retention (*e. g.*, pneumonia, cardiac incompetence, sepsis, typhoid), and the edemas observed in these diseases may possibly depend upon chloride retention, as many French authors suggest. Rumpf, indeed, often found more chlorides in edematous fluids of non-nephritic origin than in nephritic edema.

Inflammatory Edema.—Although here the alterations in the capillary walls play an essential rôle, as shown by the proteid-rich nature of the exudates, yet most of the other factors are added. Increased blood pressure is prominent; lymph outflow is impeded by plugging of the lymphatic channels by clots and leucocytes, and by pressure on the outside; there is, undoubtedly, an excessive formation of metabolic products in the tissues, to cause exosmosis. To this class of edemas belong

¹ Literature, résumé by Widal and Javal, *Jour. Physiol. et Pathol.*, 1903 (5), 1107 and 1123; also articles by Castaigne and Rathery, *Semaine Méd.*, 1903 (23), 309; Widal and Javal, *Presse Méd.*, 1903 (11), 701; Ambard and Beaujard, *Semaine Méd.*, 1905 (25), 133; Koziczowsky, *Zeit. klin. Med.*, 1904 (51), 287; Bing, *Berl. klin. Woch.*, 1905 (42), 1278; Strauss, *Zeit. klin. Med.*, 1902 (47), 337; Ferrannini, *Cent. f. inn. Med.*, 1905 (26), 1; Miller, *Jour. Amer. Med. Asso.*, 1905 (45), 1915; Rumpf, *Münch. med. Woch.*, 1905 (52), 393. Review in Albu and Neuberg's "*Mineralstoffwechsel*," Berlin, 1906, pp. 171-178.

² *Hospitalstidende*, 1904, p. 1017.

also the urticarias which follow the ingestion of various toxic substances, many of which can be shown experimentally to be lymphagogues. A good example is the urticaria which often follows the injection of antitoxic or other foreign serums, particularly their repeated injection; in experimental animals such a serum may cause death very quickly by acute pulmonary edema. All these poisons probably produce urticarial edema by injury to the capillary walls in the subcutaneous tissues—probably the other factors are not important in this condition. In the action of vesicants, however, it may well be questioned if changes in the capillary walls and active hyperemia are not supplemented by local metabolic alterations and osmotic influences.

Neuropathic Edema.—Until we understand better than we now do the manner in which nervous impulses modify metabolism, it will be difficult to estimate properly the importance of nervous impulses in the production of edema. That nervous control is a possible factor is well shown by many experiments; for example, simple ligation of the femoral vein in animals does not cause edema, but if the sciatic nerve is cut the vasoconstrictors are paralyzed, and edema may follow (Ranvier). In this case the nervous influence is only indirect, through its vasomotor effects. Similarly, stimulation of vasodilator fibers may cause edema. It is furthermore possible that nervous stimulation may lead to excessive metabolic activity, with an accumulation of crystalloidal products, sufficient to cause edema when supplemented by active congestion and some resulting pressure upon the lymph-vessels. There are certainly many instances in which edema seems to depend upon nervous disturbance; for example, edema in the area of distribution of a neuralgic nerve; sudden joint effusions in tabetic arthropathy; and especially the typical “angioneurotic” edema. The only explanation that seems open is the one given above, namely, a combination of local hyperemia and increased metabolic activity.

Hereditary Edema.—In a number of families there has been observed a peculiar inherited tendency to the occurrence of acute attacks of local edema, which not infrequently have proved fatal when involving the glottis.¹ There can be little question that these instances of hereditary edema depend upon a nervous affection of some kind, it being practically an angioneurotic edema; but how the edema is produced, and what the nature of the nervous alteration may be, are as mysterious as are most other so-called “nervous inheritances.”

¹ Literature, see Fairbanks, *Amer. Jour. Med. Sci.*, 1904 (127), 877.

COMPOSITION OF EDEMATOUS FLUIDS

As is well known, the composition of edematous fluids varies greatly according to the cause of the edema and the place where it occurs. In general, non-inflammatory edemas (transudates) contain much less proteid than do the inflammatory exudates, as is shown by the following table of analyses

TABLE I.

	Sp. gr.	Parts per 100 of fluid.			
		Total proteid.	Fibrin.	Serum-globulin.	Serum-albumin
Acute pleurisy	1.023	5.123	0.016	3.002	2.114
“ “	1.020	3.4371	0.0171	1.2406	1.1895
“ “	1.020	5.2018	0.1088	1.76	3.330
Hydrothorax } . . .	1.014	1.7748	0.0086	0.6137	1.1557
Aver. of 3 cases }					

by Halliburton¹ and by Bernheim's² determinations of proteids in ascitic fluids.

TABLE II.

Ascitic fluid in	Parts of proteid to 1000 c.c. fluid.		
	Max.	Min.	Mean.
Cirrhosis of the liver	34.5	5.6	9.69-21.06
Bright's disease	16.11	10.10	15.6 -10.36
Tuberculous and idiopathic peritonitis .	55.8	18.72	30.7-37.95
Carcinomatous peritonitis	54.20	27.00	35.1-58.96

The specific gravity varies nearly in direct proportion to the amount of proteids, that of transudates usually being below 1.015, and exudates above 1.018, although there are many exceptions. Indeed, it is often very difficult to decide whether a given fluid

¹ Adami, Allbutt's System, 1896 (1), 97.

² Quoted by Hammarsten, "Physiological Chemistry" (Amer. ed.), 1904, p. 223.

is an exudate or a transudate.¹ According to Rzentkowski,² the transudates at the moment they pass out of the vessels are simply solutions of crystalloids in water and quite free from proteid; the small amount of proteid found in transudates he ascribes to proteid pre-existing in the tissue-spaces. This idea is hardly acceptable in view of the known permeability of the vessel-walls for proteids in normal conditions; more probably in cardiac and renal dropsies the quantity of proteid escaping from the vessels is not greatly different from normal, but the excessive fluid escaping in these conditions carries with it no additional proteids, and to this extent transudates in *statu nascenti* are proteid-free.

Transudates, even when produced by the same cause, vary in composition in different parts of the body, presumably because of variations in the permeability of the vessels in different vascular areas; just as pleural, pericardial, peritoneal, and meningeal fluids normally differ from one another. Thus C. S. Schmidt³ found the composition of the transudates in different parts of the body of a patient who died of nephritis to have the following composition:

TABLE III.

	Pleural.	Peritoneal.	Subarachnoid.	Subcutaneous.
Water	963.95	978.91	983.54	988.70
Solids	36.05	21.09	16.46	11.30
Organic matter	28.50	11.32	7.98	3.60
Inorganic matter	7.55	9.77	8.48	7.70

As in this case, the general rule is that while the proportion of salts remains nearly constant, the proportion of proteid in edematous fluids in different localities varies in decreasing order as follows: (1) pleura; (2) peritoneum; (3) cerebrospinal; (4) subcutaneous. In the last-named location the specific gravity of edematous fluids may be as low as 1.005, and the

¹ Rivalta (Rif. Med., 1903; Biochem. Centr., 1904 (2), 529) has suggested the following test to distinguish exudates and transudates: Into a beaker containing 200 c.c. of water with 4 drops of glacial acetic acid, let fall a few drops of the fluid to be tested. If an exudate, a bluish-white line is left transiently behind the sinking drops, due to precipitation of the euglobulin and pseudoglobulin. Memmi (Clin. Med. Ital., 1905, No. 3) suggests the larger content of lipase as a means of distinction of exudates. Tedeschi (Gaz. degli. Osped., 1905 (26), 88) states that egg-albumen fed in large amounts appears in transudates and not in exudates, and can be detected by the biological precipitin test.

² Virchow's Arch., 1905 (179), 405.

³ Hoppe-Seyler's Physiol. Chemie, p. 607.

proteids even less than 0.1 per cent. (Hoffmann¹). An increase in solids occurs after the effusion has existed for some time, presumably because of absorption of water and salts, leaving a slowly increasing proportion of proteids. Furthermore, the composition of the patient's blood has considerable influence on the composition of the effusion; this is particularly true in the case of ascites from portal obstruction, the contents of the blood coming from the intestine during digestion modifying the composition of the ascitic fluid. Thus Müller,² in a case of portal vein thrombosis, found in the ascitic fluid of a patient on an ordinary mixed diet, 0.179 per cent. nitrogen; on a proteid-rich diet, 0.2494 per cent. N; on a proteid-poor diet, 0.1764 per cent. N. In cachectic conditions the proportion of proteids is less than in stronger individuals, and, as in the blood plasma, the albumin decreases more rapidly than the globulin as the cachexia advances (Umber³).

Physical Chemistry of Edematous Fluids.—The differences between transudates and exudates depends almost solely on their proteid contents, for the non-proteid elements are almost identical with normal lymph and blood-serum, which naturally must be so since any original or temporary deviation in osmotic pressure must be rapidly equalized by diffusion. Thus Bodon⁴ finds the concentration of the electrolytes nearly constant in spite of considerable differences in composition of various edema fluids, indicating that the serosa permits passage of inorganic salts always in the same concentration, while holding back the organic substances. Rzentkowski⁵ found some slight differences in molecular concentration as indicated by the freezing-point; in tuberculous pleurisy the average lowering was 0.523° , that of the serum being -0.56° ; in cardiac dropsy the subcutaneous fluid gave -0.548° , and in renal dropsy -0.583° ; tuberculous peritonitis, -0.523° ; cirrhosis -0.536° ; carcinomatous edema -0.547° . Of these figures, the most significant is the comparatively high molecular concentration of the fluid in nephritis, supporting the contention that the cause of renal edema is retention of crystalloids.⁶ Tieken⁷ has found the following results in transudates, exudates, and other body fluids:

¹ Deut. Arch. klin. Med., 1889 (44), 313.

² Deut. Arch. klin. Med., 1903 (76), 563.

³ Zeit. klin. Med., 1903 (48), 364.

⁴ Pflüger's Arch., 1904 (104), 519; also see Galeotti, Lo Sperimentale, 1901 (55), 425.

⁵ Loc. cit., and also Berl. klin. Woch., 1904 (41), 227.

⁶ Purulent exudates may show a high molecular concentration (-0.84° in one case), due to decomposition of the proteids into crystalloids (Rzentkowski).

⁷ Amer. Medicine, 1905 (10), 822.

TABLE IV.

Nature of Fluid.	Sp. gr.	Freezing-point of effusion, —° C.	Freezing-point of blood, —° C.	Disease.
Pleuritic effusion . . .	1,016	— 0.55	— 0.56	Pneumonia, lobar.
“ “ . . .	1,018	— 0.55	— 0.55	“ “
“ “ . . .	1,018	— 0.54	— 0.56	Tuberculosis.
“ “ . . .	1,020	— 0.55	— 0.56	“
“ “ . . .	1,016	— 0.55	— 0.56	“
“ “ . . .	1,018	— 0.64	— 0.56	Valvular heart disease.
“ “ . . .	1,030	— 0.60	— 0.58	Empyema; cyanosis.
Pericardial “ . . .	1,018	— 0.55	— 0.56	Pericarditis.
“ “ . . .	1,016	— 0.56	— 0.56	“
“ “ . . .	1,012	— 0.56	— 0.56	Hydropericardium.
Ascitic fluid . . .	1,024	— 0.60	— 0.56	Cirrhosis of liver.
“ “ . . .	1,020	— 0.57	— 0.56	“ “
“ “ . . .	1,018	— 0.58	— 0.56	Tuberculous peritonitis.
“ “ . . .	1,013	— 0.62	— 0.56	Organic heart disease.
“ “ . . .	1,035	— 0.65	— 0.58	General peritonitis.
Hydrocele fluid . . .	1,016	— 0.56	— 0.56	Tuberculosis.
Cerebrospinal fluid . .	1,018	— 0.62	— 0.58	Uremic coma.
“ “ . . .	1,016	— 0.64	— 0.68	“ “
“ “ . . .	1,020	— 0.64	— 0.64	“ “
“ “ . . .	1,014	— 0.56	— 0.56	Tuberculous meningitis.
“ “ . . .	1,017	— 0.56	— 0.56	Epidemic meningitis.
“ “ . . .	—	— 0.56	— 0.56	“ “

The very high figures for effusions in nephritis and cardiac incompetence indicate the concentration of crystalloids in these fluids, and support the belief that in the formation of both, osmotic pressure is an important factor.¹

Edematous fluids are usually alkaline except when bacterial changes lead to acid formation. Bodon² found, however, that while they contain alkali that can be neutralized by titration against acids, yet they resemble the blood in being neutral as far as the presence of free OH ions is concerned.

Proteid Contents.—As indicated in the tables given previously, these vary greatly in quantity in various fluids³; the quantitative relations of the different varieties of proteids have been less studied. Serum-albumins and globulins constitute by far the largest part of the proteids, fibrinogen being scanty except in some inflammatory exudates, so that coagulation very

¹ Meyer and His (Deut. Arch. klin. Med., 1905 (85), 149) claim that the lowering of the freezing-point is less than that of the blood in exudates while forming, the same as the blood while stationary, and greater during absorption, which they consider indicates a “vital process” on the part of the cells.

² *Loc. cit.*

³ See also v. Jaksch, Zeit. klin. Med., 1893 (23), 225; Rzentkowski (*loc. cit.*).

seldom occurs spontaneously; pneumococcus exudates seem particularly rich in fibrinogen, which coagulates rapidly and firmly. Joachim¹ found in pleural transudates and exudates that the proportion of albumin, euglobulin, and pseudoglobulin is always proportionally lower in hydrothorax than in pleurisy. Of different forms of ascites, the largest proportion of globulin and the smallest of albumin occur in cirrhosis; while with carcinoma the proportions are reversed. In general the albumin is more abundant than the globulin, but, as Umber² has found, the proportion of albumin sinks more rapidly in cachexia than does the globulin, corresponding to the similar changes in the blood proteids. The amount of proteid lost in exudates is strikingly shown by one of Umber's cases of cancerous ascites; during one year the fluid removed by paracentesis contained not less than three kilos of pure proteid, the patient weighing but 55.5 kilos.

Several authors have found in inflammatory ascitic exudates a proteid having physical and chemical properties much resembling mucin; it has been especially studied by Umber,³ who finds it quite similar to the synovial mucin isolated in arthritis by Salkowski, and calls it *serosamucin*.

Proteoses, leucin, and tyrosin may be present in small quantities in exudates, being produced by autolysis (Umber); and also mucoid substances (Hammarsten). Nucleoproteids may be present from leucocytic disintegration in exudates, as well as the products of their further splitting, such as purin bases and phosphates. Galdi and Appiani⁴ found uric acid constantly in amounts between 0.0055 g. and 0.0714 g., in all exudates, of which seven were tuberculous and two neoplastic. In three transudates amounts from 0.006 to 0.011 were found. Allantoin, which Pohl states is a characteristic product of nucleoproteid autolysis, has been found in exudates (Moscatelli⁵).

All the other innumerable components of plasma may be found in edematous fluids; thus sugar (Pickardt⁶) and urea (Carrière⁷) are usually present, as well as other extractives. Lecithin is always present, partly bound to globulin and partly

¹ Pflüger's Arch., 1903 (93), 558.

² *Loc. cit.*

³ Zeit. klin. Med., 1903 (48), 364; also Holst, Upsalalakar. Forhand, 1904, p. 304.

⁴ Riforma Med., 1904, p. 1373; also Carrière, Compt. Rend. Soc. Biol., 1899 (51), 467.

⁵ Zeit. physiol. Chem., 1889 (13), 202.

⁶ Berl. klin. Woch., 1897 (34), 844.

⁷ *Loc. cit.*

free (Christen¹). Cholesterin is present particularly in fluids that have been standing for a long time in the body, appearing often as visible crystals shining in the fluid; it probably originates from degenerating cells. Glycogen is not present (Carrière²). The various immune bodies, cytotoxins, hemolysins, bacteriolysins, agglutinins, etc., seem to pass freely into both transudates and exudates, and their presence is not characteristic of either.³

Toxicity.—Contrary to earlier ideas, transudates are not toxic, even in nephritis (Baylac,⁴ Boy-Teissier,⁵ Lafforcade⁶), and therefore the toxic manifestations frequently observed after reduction of edema in nephritis, and ascribed to absorption of poisons in the transudates, are probably due to some other cause. In inflammatory exudates, of course, the causative agents as well as the products of cell destruction render the fluids poisonous.

Enzymes.—All the enzymes of the plasma may appear in edematous fluids, being in all cases probably more abundant in exudates than in transudates. According to Carrière,⁷ oxidases are inconstant, even in exudates. Lipase is said to be much more abundant in exudates than in transudates.⁸ (Concerning proteolytic enzymes see "Autolysis of Exudates," Chap. iii.)

Precipitin Reactions, etc.—Edematous fluids have been often used as a source of material in immunizing animals against human proteids. The precipitins thus formed are specific for human serum or for the proteids of the effusion, but cannot be used to differentiate a transudate from an exudate, or a hydrothorax fluid from an ascites fluid (Quadroni⁹). Immune bodies, complement, agglutinins, and antitoxins are present in effusions¹⁰; *e. g.*, the common use of blister fluid for the Widal test. Furthermore, according to Hamburger,¹¹ edema fluid is distinctly more bactericidal than normal lymph.

¹ Cent. f. inn. Med., 1905 (26), 329.

² Compt. Rend. Soc. Biol., 1899 (51), 467.

³ Granström, Inaug. Dissert., St. Petersburg, 1905.

⁴ Compt. Rend. Soc. Biol., 1901 (53), 519.

⁵ *Ibid.*, 1904 (56), 1119.

⁶ Gaz. heb. Med. et Chir., Jan. 28, 1900.

⁷ Compt. Rend. Soc. Biol., 1899 (51), 561.

⁸ Zeri, Il Policlinico, 1903 (10), No. 11; Memmi, Clin. Med. Ital., 1905, No. 3.

⁹ Cent. f. Bakt. (ref.), 1905 (36), 270.

¹⁰ Granström, *loc. cit.*

¹¹ Virchow's Arch., 1899 (156), 329.

VARIETIES OF EDEMATOUS FLUIDS

On the preceding pages have been mentioned the chief differences in the characters of the effusions in the usual sites,¹ with their variations in proteid contents, which variation agrees with Starling's statement that the permeability of the capillary wall for proteids differs normally in different localities. Some of the other effusion fluids not mentioned previously have particular properties of some interest.

Hydrocele and Spermatocoele Fluids.—These have been studied particularly by Hammarsten,² who found the average results of analyses of seventeen hydrocele fluids and four spermatocoele fluids as follows :

TABLE V.

	Hydrocele	Spermatocoele
Water	938.85	986.83
Solids	61.15	13.17
Fibrin	0.59	
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether-extractable bodies	4.02	
Soluble salts	8.60	10.76
Insoluble salts	0.66	

Marchetti³ found in ten specimens of hydrocele fluid rather higher results for the solids than did Hammarsten. He found 57.8 to 104.2 p. m. of solids, containing organic substances 48.8 to 95.02, and inorganic substances 8.10 to 9.56; proteids, 33.5 to 90.19; ratio of globulin to albumin as 2.56 to 9.11. Among the proteids is found 1 to 4 p. m. that is not precipitated by heat. Corresponding with the analytic results, the specific gravity of hydrocele fluid is higher, 1.016 to 1.026 as against 1.006 to 1.010 for spermatocoele fluid. Cholesterin is often abundant in hydrocele fluids, appearing to the naked eye as glistening scales. Patein⁴ found sugar in most specimens of hydrocele.

Meningeal Effusions.⁵—Normal meningeal fluid differs from all other serous fluids in being clear and watery, in its low specific gravity (1.004 to 1.007), in containing but a trace of proteid which is chiefly globulin (with a trace of proteose(?)), and a reducing substance that is not sugar.⁶ Halliburton gives the following analyses of pathological accumulations of such fluids :

¹ Literature and résumé on pleuritic exudates, see Ott, *Chem. Pathol. der Tuberc.*, 1903, p. 392.

² *Physiological Chemistry*, Amer. ed., 1904, p. 223.

³ *Lo Sperimentale*, 1902 (56), 297.

⁴ *Jour. pharm. et chim.*, 1906 (23), 239; also *Compt. Rend. Soc. Biol.*, 1906 (60), 303.

⁵ *Résumé* by Blumenthal, *Ergeb. der Physiol.*, 1902 (1), 285.

⁶ Halliburton's "Chemical Side of Nervous Activity," 1901, p. 18; see also Halliburton's "Chemistry of Muscle and Nerve," 1904.

TABLE VI. (Spina bifida.)

	Case 1	Case 2	Case 3
Water	989.75	989.877	991.658
Solids	10.25	10.123	8.342
Proteids	0.842	1.602	0.199
Extractives }	9.626	0.631	3.028
Salts }		7.890	5.115

The percentage of solids in spina bifida is thus a little higher than in normal meningeal fluid. In hydrocephalus the percentage of solids is rather greater, as seen in Table VII.

TABLE VII. (Hydrocephalus.)

	Case 1	Case 2	Case 3
Water	986.78	984.59	980.77
Solids	13.22	15.41	19.23
Proteids and extractives	3.74	6.49	11.35
Salts	9.48	8.92	7.88

Normal cerebrospinal fluid seems to be hypertonic to the serum of the same animal,¹ and is much less alkaline than the blood (Cavazzani²). According to Fuchs and Rosenthal,³ the average freezing-point of the cerebrospinal fluid is lowered about the same in all diseases ($\Delta = -0.52^\circ$ to -0.54°) except in tuberculous meningitis, where it is much less (average -0.43°). The amount of potassium is usually higher than in other body fluids, according to Geoghegan the ash containing 20 to 30 per cent. of potassium salts and but 15 per cent. of sodium salts. The amount of proteid generally varies directly with the number of cellular elements present in the fluid.⁴ In diseases associated with destruction of brain tissue, such as general paralysis and epilepsy, *cholin* may be found in the spinal fluid. (See "Cholin," Chap. iv.)

Wound secretions obtained from large aseptic wounds, mostly amputation stumps, have been studied by Lieblein.⁵ The reaction is generally alkaline, globulin and albumin abundant, but fibrinogen scanty, total nitrogen being less than that of the blood and decreasing from day to day; the proportion of albumin increases and globulin decreases as healing progresses. Occasionally albumoses were found, but only on the first day in aseptic wounds; if found later, they generally were antecedent to suppuration (concerning suppuration see "Inflammation," Chap. X.).

¹ Ravaut, Presse méd., 1900 (8), 128; Zanier, Cent. f. Physiol., 1896 (10), 353.

² Cent. f. Physiol., 1902 (15), 216.

³ Wien. med. Presse, 1904 (45), 2081 and 2135.

⁴ Rénon and Tixier, Compt. Rend. Soc. Biol., 1906 (60), 639.

⁵ Beit. klin. Chir., 1902 (35), 43.

Blister fluid is generally rich in solids and proteids (40–65 p. m.). In a burn blister Mörner¹ found 50.31 p. m. proteids, among which were 11.59 p. m. globulin and but 0.11 p. m. fibrin; also a substance reducing copper oxide, but no pyrocatechin.

Chylous Effusions.²—Fat may be present in effusions in sufficient quantity to cause a milky appearance, either from escape of chyle from a ruptured or obstructed thoracic duct, or through fatty degeneration of the cells in the effusion or the lining of the walls of the cavity. The former are designated as chylous, the others as *chyliform* or *adipose* fluids, but it is not always easy to distinguish between them. The composition of the fluids in true chylous exudates will vary according to the food taken and the amount of fat the food contains, and will resemble the composition of chyle, except to the extent that it is modified by the absorption going on in the cavity.

Analyses of human chyle are scanty. The most recent are those of Panzer and of Carlier. Panzer³ found 90.29–94.53 per cent. water; 5.47–9.71 per cent. solids; 0.80–1.04 per cent. inorganic salts; 2.16 per cent. coagulable proteid; 6.59 per cent. ether-soluble material; also diastatic enzyme, soaps, and occasionally traces of cholesterin, lecithin, and sugar. Carlier,⁴ in a specimen from a child, obtained very similar results, except that the salts were much less abundant.

Edwards⁵ found but 60 definitely established cases of chylous or chyliform ascites in the literature up to 1895; and of 31 indisputable cases studied at autopsy, in 21 there was established the existence of a rupture in the thoracic duct or lacteals. Boston⁶ in 1905 was able to collect 126 cases, including both chylous and chyliform ascites, and notes an associated *eosinophilia* in a case studied by him. Chylous ascites fluid often, but not always, contains sugar,⁷ which is diagnostic if present in more than traces, and if diabetes is excluded, but it may disappear after having once been present; the amount of fat is small, usually about 1 per cent., and the fluid is rich in solids. If due to a ruptured thoracic duct, it may be possible to detect

¹ Hammarsten, Amer. ed., 1904, p. 224.

² General features reviewed by Edwards, Reference Hdbk. Med. Sci., 1901 (3), 78.

³ Zeit. physiol. Chem., 1900 (30), 113.

⁴ British Med. Jour., 1902 (ii), 175.

⁵ Medicine, 1895 (1), 257, gives literature; also see "Chem. u. morph. Eigenschaften fetthaltige Exsudaten," St. Mutermilch, Warschau, 1903; Comey and McKibben, Boston Med. and Surg. Jour., 1903 (148), 109.

⁶ Jour. Amer. Med. Assoc., 1905 (44), 513.

⁷ For example, v. Tabora (Deut. med. Woch., 1904 (30), 1595) found as high as 0.864 per cent. of sugar in a typical case.

special fats taken in the food, *e. g.*, butter-fats (Straus¹). The reaction is usually alkaline or neutral, and some specimens coagulate spontaneously. Specific gravity varies from 1.007 to 1.040, the average being about 1.017. Perhaps the most important characteristic is the variation produced by changes in diet.² Zdarek³ found in a chyle-cyst 2.7 per cent. of fats, 7.2 per cent. of proteids, and 0.05 per cent. of sugar; feeding of fats increased their amount in the cyst and starvation decreased it.

Ascites adiposus is characterized by the absence of sugar and by a higher percentage of fat, the maximum observed being 6.4 per cent. In a case examined by Edwards the composition was as follows: Specific gravity, 1.012; proteid, 2.7 per cent.; fat, 6 per cent.; diastatic ferment and sugar absent. This form occurs principally as a result of fatty metamorphosis of cells, particularly in carcinomatous and tuberculous exudates; Edwards was able to show experimentally that a transudate may change from serous to cellular, and later come to contain fat.

Pseudochylous effusions are also observed, not only in the abdominal and thoracic cavities, but even in the fluid of the edematous legs and scrotum; these resemble chylous fluids in being turbid or milky, but they contain no fat.⁴ The turbidity is apparently due chiefly to lecithin, which is largely combined with the pseudoglobulin of the fluid (Joachim⁵). Possibly in some cases the turbidity is partly or largely (Poljakoff⁶) due to poorly dissolved proteids. Strauss⁷ has noted the occurrence of this form of ascites particularly in chronic parenchymatous nephritis, but believes the turbidity has a local origin. Hammarsten has observed a similar turbidity due to mucoid substances, as also have Gouraud and Corset.⁸

¹ Arch. Physiol. et Pathol., 1886 (Ser. 3, vol. 8), 367.

² A sample of the composition of 1 liter of chylous ascitic fluid is shown by the analysis in the case studied by Comey and McKibben (*loc. cit.*): Specific gravity, 1.010; solids, 21 gm.; proteids, 9.75 gm.; urea, 1.28 gm.; fat, 1.45 gm.; inorganic matter, 8 gm.; peptone (?) and sugar, present; fibrinogen, mucin, nucleo-albumin, and uric acid absent.

³ Zeit. f. Heilk., 1906 (27), 1.

⁴ Literature, see Bernert, Arch. exp. Path. u. Pharm., 1902 (49), 32.

⁵ Münch. med. Woch., 1903 (50), 1915; also Christen, Cent. f. inn. Med., 1905 (26), 329.

⁶ Fortschr. d. Med., 1903 (21), 1081.

⁷ Note to Poljakoff's article; also Biochem. Centr., 1903 (1), 437.

⁸ Compt. Rend. Soc. Biol., 1906 (60), 23.

CHEMISTRY OF PNEUMOTHORAX

In connection with the subject of exudates the above topic may appropriately be considered. The composition of the gases found in the pleural cavity in pneumothorax will necessarily vary greatly according to the cause. If the pleural cavity is in free communication with the exterior, the gas will be simply slightly modified air; for example, Ewald¹ found the following proportions in the gases in such a pneumothorax: CO₂, 1.76 per cent.; O, 18.93 per cent.; and 79.31 per cent. N. Here the proportion of CO₂ is even a little less than in ordinary expired air, which contains 3.3–3.5 per cent. When air enters a closed pleural cavity and no effusion follows, it is slowly absorbed. At first there is a rapid absorption of oxygen, which is partly replaced by CO₂, with a resulting relative increase in nitrogen. Ordinarily the entrance of air into the pleural cavity is followed by an effusion, either serous or purulent, which may modify the composition of the gas. In a seropneumothorax Ewald found 8.13 per cent. of CO₂, 1.26 per cent. of O, and 90.61 per cent. of N, which is quite similar to the proportions of the gases in dry pneumothorax. Purulent pneumothorax generally shows more CO₂ than the serous form, the average in the former being 15–20 per cent., in the latter 7.5–11.5 per cent. The average of the analyses in six cases of pyopneumothorax is given by Ewald as 18.13 per cent. CO₂, 2.6 per cent. O, and 79.81 per cent. N. In open pyopneumothorax the gas approaches more closely the composition of air, but usually shows a slight excess of CO₂; it is thus possible by a determination of the carbon dioxide to determine quite accurately whether a given pneumothorax is in communication with the outside air. The transformation of a purulent into a putrid pneumothorax is accompanied by an increase of CO₂, even as high as 40 per cent. having been found. The products of decomposition by the putrefactive saprophytes also are present, one analysis having shown 4.3 per cent. of hydrogen, 6.25 per cent. of methane, and traces of hydrogen sulphide.

Infection of a pleural effusion by gas-producing organisms may also convert it into a pneumothorax, although this is not a common occurrence. The gases then present are the same as the organisms produce in similar culture-media, modified somewhat by absorption. The anaërobic gas-producing organisms have been found as the cause of such gaseous accumulations; it

¹ Complete literature and résumé given by Clemens, in Ott's "Chem. Path. der Tuberculose," Berlin, 1903, p. 406.

is questionable if the ordinary pathogenic organisms can cause a pneumothorax, since they are for the most part not capable of producing gas. The colon bacillus produces gas in sugar-containing media, but the amount of sugar in the pathological exudates is too small to yield any considerable amount of gas; an exception is the pleural effusion in diabetes, and pneumothorax from infection of the pleural effusion in a diabetic by *B. coli* has been reported. Complete quantitative analyses of the gas in this form of pneumothorax seem not to have been made, but May found about 20 per cent. of CO_2 . The combustibility of the gas has frequently been noted, and is probably due to hydrogen and methane.

CHAPTER XIII

RETROGRESSIVE CHANGES (NECROSIS, GANGRENE, RIGOR MORTIS, PARENCHYMATOUS DEGENERATION)

NECROSIS

WE recognize that a cell is alive through its reproducing, functioning, and its taking on and utilizing nutritive substances; yet at the same time we appreciate that a cell may do none of these things and still be alive. For example, a bacterial spore is quite inert physically, and exhibits no chemical activity, yet it is by no means dead, since it still possesses the latent power to again assume an active existence under suitable conditions. In pathological conditions we are accustomed to recognize the fact that a cell is dead by certain alterations in its structural appearance, particularly disintegrative changes in the nucleus; but this is exactly equivalent to recognizing that an animal is dead by the appearance of postmortem decomposition, for most of the characteristic histological changes of necrosis are merely postmortem changes in the cell. A cell may be dead and show absolutely none of these microscopic disintegrative changes, either because it has not been dead long enough for them to have taken place, or because the changes have been prevented by some means, just as we can prevent the appearance of postmortem decomposition by embalming. For example, if we examine microscopically the mucous membrane of the stomach of a person who has died immediately after taking a large quantity of carbolic acid, although to the naked eye this mucous membrane is hard, white, and definitely necrotic, yet we find the histological picture presented by the cells almost absolutely unchanged from the normal. The cells are dead, but they have been so "fixed" that postmortem changes could not affect their structure. All cells examined by ordinary histological methods are, of course, dead—killed by the fixing agents outside of the body, in the same way that the carbolic acid fixes them within the body. It is evident, therefore, that it may be very difficult to determine always whether a cell is dead or not. Part of the difficulty, perhaps, lies in our failure to appreciate

that not all parts of a cell die at the same time ; *i. e.*, the causes of different chemical processes of the cell reside in its different intracellular enzymes, and these are not necessarily destroyed alike by the same agents.

We recognize that after an animal is dead as a whole the various cells of its body do not die for some time, as shown by the following examples : (1) We can cause the heart to beat for a considerable period after its removal from the body ; (2) if we perfuse a mixture of glycocoll and benzoic acid through the kidney of a recently killed animal, synthesis of these substances into hippuric acid will occur ; and (3) the epithelium of the skin can be removed from the body of an animal long after death and transplanted successfully on another animal. So, too, in ordinary cell death (necrobiosis) not all the enzymes are destroyed together. When all are destroyed at once, as by strong chemicals or by heat, the customary disintegrative changes do not take place. If, however, not all the enzymes are thrown out of function, then the others may be able to act, producing the disintegrative changes by which histologists ordinarily recognize cell death. These disintegrative changes are, for the most part, apparently brought about by the intracellular proteases, that is, through autolysis. This may be shown as follows :¹ If we take two pieces of fresh normal tissue from an animal, and in one kill the enzymes by heating to 100° C., then implant both aseptically into the abdominal cavity of an animal of the same species, it will be found that the changes that follow in the two will be very unlike. In the unheated tissue nuclear changes soon occur, so that they lose their capacity for taking up basic stains, the cytoplasm becomes granular and fragmented, the tissue becomes friable so that it is difficult to secure good sections, and the changes are in general similar to those seen in areas of necrosis. The boiled tissue, on the other hand, retains its capacity for nuclear staining for months, except at the periphery, where it is slowly attacked by leucocytes and the enzymes of the blood plasma. Therefore it would seem that the characteristic changes of necrosis depend chiefly upon the intracellular enzymes, rather than upon the infiltrating plasma as Weigert² and other early writers imagined. In areas of anemic necrosis (see "Infarcts") we have another case, in which the oxidizing enzymes are thrown out of function through lack of oxygen, while the other enzymes are, presumably, at first unaffected. From studies of infarcts it would seem

¹ Wells, Jour. Med. Research, 1906 (15), 149.

² Cent. f. Path., 1891 (2), 785.

that the intracellular proteases bring about the subsequent nuclear and cytoplasmic alterations, but that the eventual digestion of the area is accomplished by the invading leucocytes working slowly inward from the periphery. Apparently when the supply of materials from outside ceases, and when the oxidation processes of the cells no longer accomplish necessary steps of synthetic reactions or destroy products of proteid catabolism, the proteases continue to split proteids without the balancing by the above-mentioned factors, with a resulting disintegration of the cells.

Karyolysis and *karyorrhexis* are, then, the result of an autolytic process, which is perhaps due to intracellular proteases that act specifically on nucleoproteids, and which may be designated as *nucleases*.¹ Nuclear staining by the usual methods depends upon an affinity of the acid nucleoproteids (in which the nucleic acid is not completely saturated by proteids) for basic dyes. Presumably in karyolysis the first step consists in a splitting of the nucleoproteid of the chromatin into nucleic acid and proteid; this can be accomplished, according to Sachs, by the ordinary trypsin, and presumably, therefore, by the trypsin-like enzymes of the cell. Corresponding with this change we should expect the free nucleic acid to give an intense staining with basic stains, and this has frequently been described by those who have studied the cytological changes in anemic necrosis,² and called *pycnosis*. As supporting this view still further may be quoted Arnheim's³ observation that in alkaline solutions the nucleus soon stains diffusely and weakly, and not at all after twelve to eighteen hours; this is to be explained by the fact that nucleic acid is both dissolved and neutralized by alkaline solutions. After the nucleic acid has been freed from the proteid by the autolytic enzymes, it is still further decomposed by the "nuclease" or similar intracellular enzymes that have the property of splitting nucleic acid into the purin bases that compose it—corresponding with this change the hyperchromatic nucleus loses its affinity for stains, and *karyolysis* is complete.

It may be observed that autolysis of aseptically preserved tissues outside the body is much more rapid than is the autolysis of infarcts and similar aseptic necrotic areas within the

¹ Jones, Amer. Jour. Physiol., 1903 (10), p. xxiv; Zeit. physiol. Chem., 1903 (41), 101; *ibid.*, 1906 (48), 110. Sachs, Zeit. physiol. Chem., 1905 (46), 337.

² Schmaus and Albrecht, Virchow's Arch., 1895 (138), supp., p. 1; Ergeb. allg. Pathol., 1896 (3), 486 (literature).

³ Virchow's Arch., 1890 (120), 367.

body. This may be due to either or both of two factors:¹ First, autolysis is much slower in alkaline than in acid media; outside the body autolyzing tissues develop an acid reaction which favors their autolysis; within the body this is checked by the alkaline plasma. Second, the plasma contains autolysis-inhibiting substances, which also may interfere with self-digestion in the body. In corroboration of the above may be recalled the fact that large necrotic areas show autolysis first in the center, where the alkaline, antagonistic body fluids presumably cause the least effect. Furthermore, it has been found by Wells² that the histological changes of autolysis proceed much faster in serum that has been heated to destroy the antibodies than in unheated serum. Leucocytes, as Opie has shown, contain autolytic enzymes acting best in an alkaline medium, hence they perform their digestive function readily at the periphery of necrotic areas.

When a cell dies, certain *physical changes* occur that are probably of considerable importance. The permeability of the cell wall is almost immediately increased, so that all diffusible substances readily pass through, *i. e.*, its semipermeable character is lost. This we see particularly in plant cells, which lose their turgor with their semipermeability, and therefore the plant wilts. Galeotti³ has studied the changes in cells that occur with their death, and finds that the electrical conductivity decreases considerably at the time of death, while the molecular concentration remains quite the same. This indicates that the number of free ions is diminished, while the number of osmotically active molecules remains constant; which Galeotti interprets as meaning that living protoplasm is characterized by a high degree of ionization. When secondary disintegrative changes occur in the protoplasm, with the formation of many small molecules from the large molecules of the cell, both osmotic pressure and electrical conductivity increase rapidly.

CAUSES OF NECROSIS

Anemia.—After the cutting off of blood-supply, cells soon undergo morphological changes that we recognize as indicating their death, and after a time they also become incapable of returning to their normal condition when the blood-supply is re-established, probably because of these structural changes.

¹ Literature and more complete discussion under "Autolysis."

² Jour. Med. Research, 1906 (15), 149.

³ Zeit. f. Biol., 1903 (45), 65.

In just what way lack of nourishment causes death has not been determined, but, as has been before suggested, it seems probable that it is because catabolic processes are no longer balanced by anabolic processes, and with these latter oxidizing enzymes seem to be inseparably associated, so far as our present knowledge shows us. Were it not that the proteolytic enzymes continue in action after nutrition is shut off, the cells might remain in a completely unaltered condition for an indefinite period, and capable of resuming their functions when nourishment is again supplied, which is decidedly contrary to the facts. (The general features of anemic necrosis have been already discussed in the preceding paragraphs, and also under the subject of infarction.)

Thermic Alterations.—These have been studied particularly in connection with the cells of the lower organisms.¹ While some unicellular organisms can survive a temperature of 69°, most of them are killed at from 40°–45°. For the great majority of metazoa the maximum temperature lies below 45°, and in the case of marine species below 40°.² The heating is accompanied by the appearance of granules in the cytoplasm, which become larger until the condition of “heat rigor” sets in. Kühne, in 1864, showed that in muscle cells, at least, there is contained a proteid which becomes turbid through partial coagulation at 40°, and Halliburton³ has found that in nearly all tissues are globulins coagulating at from 45°–50°; it is probable, therefore, that the granules formed in heated cells are produced through coagulation of these proteids. The importance of this coagulation in determining death is not yet fully established, but it would seem to be very great. Halliburton has observed that in both muscles and nerves to which heat is applied, contractions occur at various temperatures, corresponding exactly with the temperatures at which the several varieties of the proteids of the cell coagulate. Furthermore, Mott⁴ has found that the temperature that is immediately fatal to mammals (47°) is exactly the same as the coagulating temperature of the lowest coagulating proteid of nerve-cells. This fact is undoubtedly of

¹ Literature, see Davenport, “Experimental Morphology,” New York, 1897; Schmaus and Albrecht, *Ergebnisse der Pathol.*, 1896 (3, Abt. 1), 470.

² The adaptation of animal cells to high temperatures is an interesting topic, especially in view of such results as those of Dallinger, who, by raising the temperature gradually during several years, caused flagellata with a normal maximum of about 21°–23° to become capable of living at 70° (see Davenport).

³ “Biochemistry of Muscle and Nerve,” Phila., 1904.

⁴ Quoted by Halliburton.

great practical importance in causing death from fever, for although 47°C . (117°F .) is probably never reached in man, yet application of much lower temperatures, even 42° (108°F .), for a few hours will cause coagulation of these proteids (all proteids coagulate at less than their ordinary coagulation point if the heating is continued for a long time). It would seem from the above observation that heat causes cell death through coagulation of the proteids. Whether the cell death is in any way dependent upon destruction of the enzymes by heat has not been ascertained; but as most enzymes are not destroyed much below 60° – 70° , it seems improbable that they are greatly injured at the temperatures at which cells are killed. It is possible, however, that under the conditions in which enzymes exist in the cell they may be more susceptible to heat than under normal conditions. Just how coagulation of cell globulins can determine the death of a cell is difficult to understand, unless the physical conditions of the cell are greatly altered thereby. Ordinarily we have in the cell an equilibrium between colloids in solution and colloids in the solid or gel state; if the colloids are rendered insoluble by heat, so that this equilibrium is destroyed, serious alterations in the mechanism of all metabolism must result (Mathews).

Different tissues show unequal susceptibility to heat. Werhovsky¹ found the blood most affected by raising the temperature of living animals, next the liver, kidneys, and myocardium in order, the other tissues being little or not at all structurally injured.

Cold is well withstood by unicellular forms, and relatively poorly by more complex organisms, particularly by those with a highly developed circulatory system; this is because individual cells are not greatly affected by freezing, whereas the circulatory channels are readily blocked by this cause. Bacterial cells are not killed by exposure for long periods to the temperature of liquid air² (-190°). Reduction of the temperature of plant cells to -13° may result in a granular transformation of the cytoplasm, often with rather serious structural alterations. Cytoplasm seems to be more affected than the nucleus, for mitosis may occur slowly in plant cells at -8° , and Ushinsky³ noted that in animal tissues the nuclei were less affected by cold than the cytoplasm. Blood seems little affected by freezing temperature, for du Cornu found that dog's blood kept on ice for five to ten days could be

¹ Ziegler's Beitr., 1895 (18), 72.

² MacFadyen, Lancet, 1900 (i), 849.

³ Ziegler's Beitr., 1893 (12), 115.

employed for transfusion without causing hemoglobinuria. Grawitz saw motion persist in human ciliated epithelium kept for seven to nine days on ice. Ciliated epithelium from the mouth of the frog may survive cooling to -90° , and frog eggs are not killed by -60° . In many cells, however, the physical changes produced by freezing, and also by the subsequent thawing, are sufficient to render them incapable of further existence. Cells devoid of or poor in water cannot be killed by freezing, hence it is probable that the currents set up about the crystals of ice in thawing, as well as the rapid contraction and expansion under the influence of the cold and the ice formation, are the cause of the effects of freezing, which, therefore, are not dependent upon chemical, but upon physical, alterations.

In the case of warm-blooded animals, the gangrene following freezing depends not so much upon the freezing of the cells themselves as upon the formation of hyaline thrombi in the injured vessels (v. Recklinghausen, Hodara¹). Kriege² found that if the freezing is transitory, the thrombi may again disappear; if over two hours in duration, they are persistent. Rischpler,³ however, considers that cell death is due primarily to the effect of the cold upon the cells.

Light.—Light may affect tissues seriously, apart from the effects of accompanying heat. In the treatment of lupus by the Finsen method with concentrated light rays, the action is largely a stimulating one, but associated with or subsequent to a certain degree of cell injury. Ogneff⁴ found that moderate action of electric light, rich in violet and ultraviolet rays, causes mitotic cell division; if the action is stronger, the cells undergo amitotic division and then become necrotic. The destruction of bacteria by light is a well-known phenomenon, but it has been suggested that their destruction depends rather upon the action of substances produced in the culture-medium under the influence of light than upon the effect of the light upon the bacterial cells themselves. In view of the fact that enzymes in solution are quite readily weakened or destroyed by the action of light, it is possible that intracellular enzymes may be similarly destroyed by light, with resulting cell death. However, in the case of bacteria, at least, the effects of light seem to depend upon oxidation processes, for in the absence of oxygen, bacteria are not seriously injured by light, and D'Arcy and Hardy⁵ found that "active oxygen" is formed by the same

¹ Münch. med. Woch., 1896 (43), 341.

² Virchow's Arch., 1889 (116), 64.

³ Ziegler's Beitr., 1900 (28), 541.

⁴ Pflüger's Arch., 1896 (63), 209.

⁵ Jour. of Physiol., 1895 (17), 390.

portion of the spectrum that is most active in destroying bacteria. Whether oxidative processes are the cause of death in animal cells is not known, but we are familiar with many chemical reactions of various sorts that are initiated or checked by the action of light.¹ Thus, bilirubin is oxidized into biliverdin, when acted upon by sunlight, even when not in contact with air; many vegetable oils are oxidized by sunlight, and it is probable that the oxidizing action of light upon organic compounds is of wide-spread occurrence. It is, therefore, quite possible that such oxidative changes may be the cause of necrosis produced by the action of light rays.

x-rays produce necrosis which is peculiar in that an interval of several days, or even weeks, may elapse after the exposure before the necrosis manifests itself. Ellis,² who has studied the literature, considers that the amount of necrosis is out of proportion to the changes in the vessels, which some have believed to be the cause of *x-ray* gangrene, and therefore that the cells must be directly injured.³ That *x-rays* have a marked effect on metabolism has been abundantly established. According to Musser and Edsall,⁴ the effect of *x-rays* upon metabolism is unequalled by any other therapeutic agent, and is manifested by excessive elimination of the products of proteid destruction, which arise particularly from the lymphatic structures.⁵ These changes have been studied, therefore, particularly in connection with the treatment of leukemia (*q. v.*). The renal epithelium seems also to suffer injury in some cases.⁶ *Radium*, which shares with *x-rays* the power of causing tissue necrosis, does not have a similar effect upon the blood, nor do the ultra-violet rays (Linser and Helber⁷).

The long-continued action of *x-rays* upon the skin has, in many cases, led to the formation of cancer, apparently because the proliferation stimulated by the rays progresses until it exceeds normal bounds.⁸

As the metabolic changes produced by *x-rays* indicate an

¹ See Davenport, "Experimental Morphology," 1897, p. 162.

² Amer. Jour. Med. Sci., 1903 (125), 85.

³ Allen (Jour. Med. Research, 1903 (9), 462) states that protozoa and vinegar eels are killed by long exposure to *x-rays*, whereas plants are decidedly stimulated in their growth.

⁴ Univ. Penn. Med. Bull., 1905 (18), 174.

⁵ A peculiar selective action for the generative cells is also shown by *x-rays*, which cause marked atrophy of the ovaries and testicles. (See Albers-Schönberg, Münch. med. Woch., 1903 (50), 1859; Friebe, *ibid.*, 1903 (50), 2295; Specht, Arch. f. Gyn., 1906 (78), 458; Thaler, Deut. Zeit. Chir., 1905 (79), 576.

⁶ See Schulz and Hoffman, Deut. Zeit. f. Chir., 1905 (79), 350.

⁷ Deut. Arch. klin. Med., 1905 (83) 479.

⁸ See review by Wyss, Beitr. z. klin. Chir., 1906 (49), 185.

extremely high rate of autolysis, one may ascribe the effects either to a stimulating effect of x -rays upon autolytic enzymes, or, as Neuberg¹ does, to an inhibitive action of x -rays and radium rays upon the other intracellular enzymes without a corresponding deleterious effect upon the autolytic enzymes. This hypothesis agrees with the facts at hand, but more details concerning the effects of these rays upon various enzymes are needed. The long latent period before the appearance of necrosis after exposure to x -rays is difficult to explain, and agrees rather with the hypothesis of slow proliferative and obstructive changes in the blood-vessels.

Electricity.—The effects of the electric current upon cells are described by Davenport as follows: A weak constant current causes a centripetal flowing of the protoplasm (in *Actinosphaerium*); if the current is increased or long continued, the cytoplasm of the pseudopodia becomes varicose, and droplets are formed which soon burst, causing a collapse of the protoplasmic framework. Finally, the protoplasm on the anode side begins to disintegrate, and the loose particles move toward the positive electrode; eventually the cell structure may be entirely destroyed. If an alternating current is used, both anode and cathode side of the cell are affected. In moving organisms electric currents determine direction of motion, even certain vertebrates (tadpoles, fish) being made to orient themselves according to the current. The nucleus seems to be more susceptible to harm by electric currents than the cytoplasm (Pfeffer²), and there seems to be no oxidation-process involved in cell destruction by electricity (as is the case with light rays), for the effects are much the same in the absence of oxygen (Klemm). Schmaus and Albrecht state that the effect of electricity upon protoplasm depends upon a loosening of the cohesion and a solution of the constituents of the cell (vacuolization), which last is, perhaps, due to direct chemical alterations. It may be suggested that the electric current causes a migration of ions toward one or the other pole of the cell, in this way separating the movable inorganic ions of the ion-proteid compounds of the cell from the immobile colloidal proteid ions, with consequent serious alterations in the chemistry of the cell. Zeit³ found that continuous currents kill bacteria through the production of antiseptic substances in the culture-medium, but do not harm them directly.

¹ Zeit. f. Krebsforschung, 1904 (2), 171.

² Literature given by Davenport, "Experimental Morphology."

³ Jour. Amer. Med. Assoc., 1901 (37), 1432, literature.

Jellinek¹ has studied extensively the cause of death after severe electric shocks, and finds that there are produced intracerebral hemorrhages and degeneration of the nerve-cells, which are sufficient to explain the death of the individual without having recourse to the more indefinite idea of "shock." Cunningham² considers fibrillary contraction of the heart as the cause of death.³

Chemicals cause cell death whenever they are of such a nature as to either coagulate the cell proteids or to destroy its enzymes. The action of such substances as sulphuric acid, strong caustics, etc., hardly calls for explanation. Phenol (carbolic acid) may cause necrosis and gangrene even when in very dilute solutions; this appears to be due more to the production of hyaline thrombi of agglutinated red corpuscles in the capillaries than to direct action upon the cells. In some unpublished experiments on the subject of "carbolic acid gangrene," I found this action of phenol very striking when dilute solutions were placed on the web of a frog's foot, under the microscope; as soon as the solution penetrated to a capillary, stasis with fusion of the corpuscles occurred in a very few seconds. Similar results have been obtained by Rosenberger.⁴ Some poisons seem to cause necrosis without destroying the autolytic enzymes, in which case the cells are rapidly digested; at least, such a hypothesis seems best to explain the changes seen in the liver in chloroform poisoning, acute yellow atrophy, eclampsia, etc.⁵ Not all poisons, by any means, cause cell death—tetanus toxin, morphine, and other alkaloids cause death of the individual as a whole without usually causing primary necrosis of any of the cells. Cell death does not necessarily depend upon destruction of *all* the cellular enzymes, as has been pointed out previously. Thus, bacteria may be killed by many chemicals which seem not to affect their autolytic enzymes seriously.

The term, "protoplasmic poison," has been variously used and defined. Kunkel says that a protoplasmic poison "is a poison which, without producing directly evident alterations, harms or kills all living protoplasmic structures." HgCl_2 is such a poison, whereas H_2SO_4 , bromine, and similar substances that destroy all life through their strong chemical action are not included in this category. The protoplasmic poisons presum-

¹ Virchow's Arch., 1902 (170), 56; Lancet, 1903 (i), 357.

² New York Med. Jour., 1899 (70), 581.

³ Full discussion by Jelliffe in Peterson and Haines' "Legal Medicine and Toxicology," 1903 (1), 245.

⁴ Verh. Phys. Med. Gesellsch. z. Würzburg, 1900, vol. 34.

⁵ Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

ably act by combining with one or more of the constituents of cell protoplasm; *e. g.*, HgCl_2 probably combines with the proteids, chloroform with the cell lipoids (physically?). Kunkel suggests that oxalic acid and fluorides are poisons because they combine the cell calcium, and barium salts may be poisonous because they precipitate the SO_4 ions. We can readily imagine that the combining of even one of the essential constituents of the cell may so upset the normal chemical processes that the cell can no longer take up substances to repair its waste, and hence necrosis ensues.¹

Physical agents may cause necrosis, usually in ways too obvious to require explanation. With most cells, large portions of the cytoplasm can be destroyed without serious results, for so long as the nucleus is intact the cytoplasm can be reconstructed. The fact that necrosis frequently follows relatively slight injuries of the nucleus is perhaps best explained by considering that injury to the nuclear membrane modifies the permeability of the nucleus for substances in solution, which might readily affect its metabolic activities to a serious degree. It is possible, also, that solvents of lipoids, such as chloroform, etc., produce much of their deleterious effects by modifying the permeability of the cell, since the semipermeability of cell membranes depends largely upon the lipoids they contain.²

Physical injury of even slight degree may bring on severe alterations in cells, however, and indeed may cause severe chemical alterations. We know that many chemical reactions can be brought about by slight mechanical disturbances; *e. g.*, the explosion of fulminate, nitrogen iodide, etc., and it is quite possible that mechanical disturbances can, likewise, cause chemical changes in the protoplasm. Many lower animals devoid of a nervous system respond to mechanical stimuli by chemical activity; *e. g.*, the production of phosphorescence by marine organisms when agitated by an oar, etc. Possibly, the secretion of thrombokinase by the leucocytes, which occurs whenever they come in contact with a foreign body, is an example of a similar reaction to a mechanical stimulus. We have no good evidence, however, that mere contact with a chemically inert foreign body, unaccompanied by cellular injury, can cause death of tissue-cells.³

¹ It is hardly profitable here to go further into the theories of the action of poisons, which are generally extensively considered in the treatises on toxicology and pharmacology (also by Davenport, *loc. cit.*).

² See Pascucci, Hofmeister's Beiträge, 1905 (6), 552.

³ Meltzer (Zeit. f. Biol., 1894 (30), 464) has shown that bacteria may be killed by violent agitation, which causes disintegration of the cells.

Extreme changes in osmotic pressure may lead to cell death, either by causing structural alteration in the cell (*e. g.*, the bursting of plant-cells in water), or concentration of the electrolytes may become so great that the colloids are thrown out of solution, as in the ordinary salting-out processes of the laboratory. It is doubtful, however, if osmotic changes *per se* ever become so abnormal within the animal body (except in experimental conditions) as of themselves to cause cell necrosis.

VARIETIES OF NECROSIS

Coagulation Necrosis.¹—This name is applied to necrotic areas that are firm, dry, usually pale yellowish in color, and observed principally in areas of total anemia or tuberculosis. The question has been long disputed as to whether a true coagulation occurs in such tissues or not. Necrosis produced by heat, carbolic acid, corrosive sublimate, etc., is naturally a coagulation necrosis, the cells of the affected area having undergone true coagulation; *i. e.*, the conversion of their soluble colloids (*sols*) into the insoluble "*pectous*" modification. Whether the same change occurs in areas of anemic necrosis is not so well established. If the part contains a fair amount of plasma the liberation of the tissue coagulins from the dead cells will cause a conversion of the fibrinogen into fibrin—this can usually be demonstrated microscopically, but the presence of fibrin is not constant, and its quantity is usually insufficient to explain satisfactorily the condition of coagulation necrosis in infarcts, etc., as Weigert maintained.² Schmaus and Albrecht believe that a true coagulation of the cell proteids does occur in anemic infarcts, etc., for they found that the cells of kidneys with ligated vessels contain at first granules soluble in water and salt solution; after forty-eight hours the granules cannot be dissolved in these solvents or in weak acetic acid, but are soluble in 2 per cent. KOH; after five to six days the granules are insoluble even in KOH. Beyond these experiments, we seem to have no proof of the occurrence of intracellular coagulation within areas of coagulation necrosis due to anemia; exact chemical studies on this point are much needed. Since tissue-cells contain

¹ Literature by Jores, *Ergebnisse der Pathol.*, 1898 (5), 16.

² Weigert believed that the dead area becomes permeated by plasma containing fibrinogen, which is coagulated in and between the cells. He put much weight on an increase in size of the necrotic area, which is by no means constant, as he intimated; necrotic areas are inelastic, and when death occurs, they do not shrink with the fall of blood pressure as the surrounding tissues do, and hence they may appear to project from the surface of the dead organ when they did not do so during life.

coagulins for fibrinogen, it is possible that they also contain coagulins for cell-proteids, but this remains to be established. Bacteria produce substances coagulating milk and fibrinogen, and Ruppel¹ found that the tubercle bacillus produces substances precipitating proteids; hence coagulation necrosis in bacterial infections may be brought about in this way; and Schmoll² has shown that the necrosis occurring in tubercles is associated with an almost complete coagulation of the cell-proteids.

Necrosis associated with inflammatory exudation is, of course, accompanied by coagulation of the fibrinogen of the exudate (*e. g.*, diphtheria); this type of coagulation necrosis is chemically a simple fibrin-formation and readily understood. The peculiar hyaline degenerations of parenchymatous cells (*e. g.*, Zenker's degeneration of muscles) are often included under this class, but it would seem more probable that the processes consist rather of the fusion of the structural elements of the cell into a homogeneous substance than a true coagulation. No exact data are at hand concerning this point, however.

Liquefaction necrosis occurs particularly in the central nervous system, where the cell substance seems not to undergo the coagulative changes described in the preceding paragraphs. Whether this is due to a lack of tissue-coagulins or to a difference in cell composition cannot be said, but the large proportion of lipoids in brain tissue is probably an important factor. Probably "*edema ex vacuo*" is responsible for much of the accumulation of fluid, due to the anatomical conditions that prevent a shrinking or collapse of the tissues to fill in the gap, and the lack of connective-tissue formation. Aseptic softening in general may be safely ascribed to digestion of proteids by cellular enzymes, either from the dead cells or from the leucocytes. Suppuration is merely a form of liquefactive necrosis, in which such digestion is particularly rapid because of the large number of leucocytes that are present. Necrosis of the gastric mucosa or of the pancreas is also followed by rapid liquefaction, through the action of the digestive enzymes of these tissues. When necrosis is accompanied by edema (as in superficial burns), the fluid enters the cells in large amounts, presumably because of increased intracellular osmotic pressure, and in this way another form of liquefaction necrosis may be produced.

Caseation.—This term is applied to a form of coagulation necrosis in which the dead tissue has an appearance quite similar

¹ Zeit. physiol. Chem., 1898 (26), 218.

² Deut. Arch. klin. Med., 1904 (81), 163.

to that of cheese. If we bear in mind the fact that cheese is a mixture of coagulated proteid and finely divided fat, and that in caseation we have a coagulation of tissue proteids associated with the deposition of considerable quantities of fat, the reason for the gross resemblance of the product of this form of necrosis to cheese is apparent. Schmoll¹ has analyzed caseous material, and found it almost entirely free from soluble proteids or proteoses. The proteid material is almost solely coagulated proteid, which in its elementary composition is related to the simple proteids or to fibrin, and not at all to the nucleoproteids. The extremely small amount of phosphorus present in the caseous material indicates that the products of disintegration of the cell-nuclei must diffuse out early in the process. Caseation is, therefore, characterized by a coagulation of the proteids and a dissolving out of the nuclear components. Schmoll does not explain the cause of coagulation, however. It may be that it is the same as in the coagulation of anemic infarcts (since tuberculous areas are decidedly anemic), or possibly the tubercle bacillus produces substances coagulating proteids, as Ruppel² states is the property of "tuberculosamin." Indeed, Auclair³ claims that the fatty substance that can be extracted from tubercle bacilli by chloroform is the cause of the caseation. Dead tubercle bacilli do not produce true caseation, however, according to Kelber⁴; hence the substance causing the necrosis evidently does not diffuse readily from the bodies of the bacilli.

The abundance of fat in caseous material is very striking. Bossart⁵ found from 13.7 per cent. to 19.4 per cent. of the dry substance of caseous material soluble in alcohol and ether. In the scrapings from tuberculous bovine glands I have found 22.7–23.9 per cent. of the organic material soluble in alcohol and ether.⁶ Of this soluble material, Bossart found 25 to 33 per cent. of cholesterin, and Leber⁷ found 38.31 per cent. of lecithin, which is a much higher proportion than Bossart detected. Presumably these fatty materials are derived chiefly from the disintegrated cells; this is probably true of the lecithin and cholesterin, but the fact that in histological preparations most of the fat is found about the periphery of the caseous area,⁸

¹ Deut. Arch. klin. Med., 1904 (81), 163.

² *Loc. cit.*

³ Arch. méd. exper., 1899, p. 363.

⁴ Quoted by Dürk and Oberndorfer, *Ergebnisse der Pathol.*, 1899 (6), 288.

⁵ Quoted by Schmoll, *loc. cit.*

⁶ Wells, *Jour. Med. Research*, 1906 (14), 491.

⁷ Quoted by Schmoll.

⁸ Sata, *Ziegler's Beitr.*, 1900 (28), 461.

supports the belief that it has wandered in from the outside.¹ A certain proportion of the fat is probably derived from the bodies of the tubercle bacilli, which usually contain about 40 per cent. of fatty matter; but it has not been determined whether the fat from this origin forms an appreciable part of the fatty matter of caseous material.

Caseous areas persist for extremely long periods of time without undergoing absorption, which indicates that the autolytic enzymes are destroyed early in the process, presumably by the toxins of the tubercle bacillus; corresponding to this Schmoll found autolysis very slight indeed in caseous areas. Because of a lack of chemotactic substances no leucocytes enter to remove the dead material. That the failure of absorption is not due to a modification of the proteids into an indigestible form is shown by the rapid softening of caseous areas when, through mixed infection, chemotactic substances are once developed and leucocytes enter.

FAT NECROSIS.²

Through usage this term has come to indicate a specific form of necrosis of fat tissue, which is characterized by a focal, circumscribed arrangement, and by the splitting of the fat in the necrotic area into fatty acids and glycerin, the latter disappearing, the former combining with bases to form soaps.³ In all cases fat necrosis is produced by the action of pancreatic juice upon fat tissue,⁴ presumably through the action of the enzymes it contains, and the condition can be produced experimentally by any procedure that causes escape of the pancreatic juice from its natural channels.

Langerhans⁵ made the first studies of the nature of the changes in fat necrosis, and established the fact that the fat of the cells is split into its components, and that the fatty acids combine (at least in part) with calcium. Dettmer⁶ found that,

¹ Fischler and Gross (Ziegler's Beitr., 1905 (7th suppl.), 344) could find no fatty acids in caseous areas by histological methods.

² General literature will be found in the articles cited in the text; also in Opie's "Diseases of the Pancreas," 1903; and in Truhart's "Pankreas-Pathologie," Wiesbaden, 1902.

³ The fatty acids form masses of crystals in the fat-cells, and they can also be demonstrated microchemically by Benda's method (Virchow's Arch., 1900 (161), 194), which consists of staining with a copper acetate mixture, blue-green copper salts of the fatty acids being formed.

⁴ Wulff (Berl. klin. Woch., 1902 (39), 734) claims to have observed an exception to this rule, but his account is not by itself convincing.

⁵ Virchow's Arch., 1890 (122), 252.

⁶ Dissertation, Göttingen, 1895.

although fresh pancreatic juice caused fat necrosis, a commercial preparation of trypsin did not do so, and, therefore, he concluded that probably the lipase of the pancreatic juice was the active agent. Flexner¹ supported this contention by demonstrating the presence of a fat-splitting enzyme in foci of fat necrosis, which was corroborated by Opie.² The latter³ was also able to demonstrate the presence of lipase in the urine of a patient with fat necrosis.⁴

In a study of the pathogenesis of fat necrosis, particularly with reference to the question whether the lipase or the trypsin of the pancreatic juice was responsible, Wells⁵ found that typical fat necrosis could be produced by injecting extracts of fresh pancreas into animals, either of the same species as that from which the pancreas was obtained, or into a foreign species. Commercial "pancreatins" were also quite effective, whether in weak acetic acid or weak alkaline solutions. The power of these materials to cause fat necrosis was reduced by heating to or above 60° for five minutes, and completely destroyed at 71°, indicating that the active agent is an enzyme. But, as in the same material trypsin was injured by temperatures above 60°, and destroyed at between 70° and 72°, and lipase was weakened above 50°, and destroyed above 70°, it was impossible to determine, by heating pancreatic preparations, whether the lipase or the trypsin was the essential factor. By permitting pancreatic extracts to digest themselves it was found that the power to produce fat necrosis decreased, *pari passu*, with the decrease in lipolytic strength. Preparations strongly tryptic, but very weak in lipase, produced no fat necrosis, and, on the other hand, extracts of pig's liver or of cat's serum, both rich in lipase but devoid of trypsin, were equally ineffective. Furthermore, mixtures of liver or serum lipase and trypsin were incapable of causing fat necrosis. Fresh pancreatic extracts from fasting dogs, containing lipase but almost no trypsin (which in fresh extracts is still in the form of inactive trypsinogen), produced abundant fat necrosis, whereas after the trypsinogen in such extracts was activated by enterokinase, no fat

¹ Jour. Exper. Med., 1897 (2), 413.

² Contrib. of pupils of W. H. Welch, Baltimore, 1900, p. 859; Johns Hopkins Hosp. Rep., 1900 (9), 859.

³ Opie, "Diseases of the Pancreas," Lippincott, 1903, p. 156; Johns Hopkins Hosp. Bull., 1902 (13), 117.

⁴ It yet remains to be seen if this is a constant occurrence; and also if the lipase so excreted comes from the pancreas, for Zeri (Il Policlinico, 1905 (12), 733 has found lipase in the urine in hemorrhagic nephritis and inflammation of the urinary tract.

⁵ Jour. Med. Research, 1903 (9), 70.

necrosis could be produced. It therefore seems certain that trypsin alone cannot produce fat necrosis, and that the decrease in strength of lipase in a pancreatic extract is associated with a corresponding decrease in power to produce fat necrosis. But, on the other hand, lipase of liver or blood-serum alone, or when mixed with trypsin, will not produce fat necrosis. The possibility remains that pancreatic lipase is different from liver or serum lipase, and can by itself cause fat necrosis; more probably, however, the production of fat necrosis depends upon a double action, trypsin causing the death of the cells, and lipase splitting the fats.¹ The fatty acids alone will not cause necrosis of fat-cells, and it was shown that the first steps in the process consist of a necrosis of the surface endothelium extending into the connective and fat tissue; this may occur in a few minutes, while evidence of fat-splitting can be obtained only after about three hours, and the splitting occurs only in cells that have already become necrotic; hence the fat-splitting is not the cause of the necrosis, but occurs subsequent to the necrosis. After about four hours a substance appears in the decomposed fat that stains with hematoxylin, which is probably calcium.

Fat necrosis may be produced by any means that will cause the escape of pancreatic juice from the natural channels within the gland. In human pathology it has followed trauma and acute infection of the gland, but the most common cause is probably the blocking of the ampulla of Vater by gall-stones, which permits the bile to back up into the pancreatic duct, where it produces an acute inflammation of the pancreas (Opie²). Flexner³ has shown that it is the bile salts that cause the inflammation, and also that this effect is decreased or prevented by the presence of large amounts of colloids. As a result of injury by bile salts, or any other agent that produces cell death, the dead and injured cells are digested by the pancreatic juice which then makes its escape into the surrounding fat tissue. Wells' experiments showed that the lesions of fat necrosis may be pro-

¹ When fat tissue dies in the body from other causes, the lipase normally contained within the fat tissue does not cause the changes seen in fat necrosis. It is possible, therefore, that the combining of newly split fatty acids by the alkali of the pancreatic juice is responsible for the formation of the large amount of soaps found in fat necrosis. Otherwise we might expect the lipase to produce only an equilibrium, and that, in the case of fat, seems to exist when most of the substance is neutral fat. In support of this idea I found that strong alkalis injected into fat tissue sometimes caused changes very closely resembling areas of fat necrosis in the early stages.

² Bull. Johns Hopkins Hosp., 1901 (12), 182.

³ Jour. Exp. Med., 1906 (8), 167.

duced in three to five hours, large enough to be visible to the naked eye; their form and size depend solely upon the area of fat tissue exposed to the action of the pancreatic juice. The process progresses for but a few hours, the extension seeming to be limited by surrounding leucocytes. The lesions may appear at remote points in the thoracic and pericardial cavities or in the subcutaneous tissues, the causative agent probably being carried by the lymphatic vessels. Fat necrosis itself is not dangerous to the affected organism, the associated pancreatitis (and peritonitis) causing all the symptoms.¹ There is no evidence that sufficient quantities of soaps (which are toxic) are absorbed from the necrotic areas to cause appreciable intoxication. Apparently, however, glycerine is absorbed in sufficient quantities to appear in the urine, for on this basis Cammidge² has devised a method of diagnosis of pancreatic lesions by examining the urine for glycerin, the value of which Robson³ has affirmed. Healing follows rapidly in case of recovery; the foci may disappear as early as eleven days after their formation (in experimental animals).

Self-digestion of the pancreas occurs soon after death, and the pancreatic juice may in this way bring about a postmortem fat digestion that resembles somewhat the intravital fat necrosis in its gross appearances,⁴ and Wells found that the same changes might be produced by injecting pancreatin into the bodies of dead animals, or by keeping fat tissue in pancreatin solutions. Wulff found that fatty acids were demonstrable by Benda's method in the pancreas of nearly all cadavers. The process differs from the *intra vitam* form in being less sharply circumscribed, and microscopically by the absence of cellular and vascular reaction. That the essential changes of fat necrosis can be produced postmortem is final proof that they are due to enzymes, rather than to circulatory or cellular action.

¹ Guleke (Arch. klin. Chir., 1906 (78), 845) considers the intoxication of acute pancreatitis as an intoxication with trypsin, which can be checked by antitrypsin. Doberauer (Beitr. klin. Chir., 1906 (48), 456), however, looks upon the products of cellular disintegration as the source of the intoxication. v. Bergmann (Zeit. exp. Path. u. Ther., 1906 (3), 401) states that the toxicity is not due to either the enzymes or to albumoses; and that it is a true auto-intoxication which can be prevented by previous immunization with either pancreas extracts or commercial trypsin.

² Brit. Med. Jour., 1904 (i), 776; Lancet, 1904 (i), 782; 1906, May 19.

³ Lancet, 1904 (i), 779.

⁴ Chiari, Zeit. f. Heilk., 1896 (17), 69; Pförringer, Virchow's Arch., 1899 (158), 126; Liepmann, *ibid.*, 1902 (169), 532; Wulff, Berl. klin. Woch., 1902 (39), 734.

GANGRENE

This term indicates merely that certain marked secondary changes, either putrefaction or desiccation, have occurred in necrotic areas of some size. Hence we have the chemical changes of putrefaction added to those of necrosis in the case of moist gangrene, whereas in dry gangrene nearly all the chemical changes are brought to a standstill through the desiccation. In the latter it is only at the line of demarcation, where some moisture remains, that chemical changes still go on; these consist chiefly of autolysis of the dead tissues, and also of their digestion by leucocytes, which results eventually in the separation of the dead tissue from the living; this is best seen after surface burns, carbolic-acid gangrene, etc.

Moist gangrene is accompanied by the dual action of the cellular enzymes and of the putrefactive organisms that are growing in the dead tissue, and as a result such tissue contains all the innumerable products of the decomposition of proteids and fats. Thus Ziegler mentions as morphological elements that may be present in gangrenous tissues: Fat-needles, the so-called "margarin" crystals (a mixture of stearic and palmitic acids), fine acicular crystals of tyrosin, globules of leucin, rhombic plates of triple phosphate, black and brown masses of pigment, and crystals of hematin. In the sputum from pulmonary gangrene crystals of fatty acids are a peculiarly characteristic feature, and according to Schwartz and Kayser,¹ they are produced by the action of bacteria upon fats, rather than by the lipolytic enzymes of the tissues themselves. In solution we also have, beyond a doubt, all the substances formed in the decomposition of proteids, from proteoses and peptones down through the different amino-acids to such final products as ammonia and its salts, while CO_2 and H_2S are abundantly given off. In addition occur, undoubtedly, many of the ptomaines which are formed by the action of the bacteria upon the amino-acids derived from the proteids.²

If the necrotic tissue is in contact with living tissue over a considerable area, enough of these products of autolysis and putrefaction may be absorbed to cause intoxication (*sapremia*). At the same time, the formation of such large quantities of crystalloids from the proteids of the dead tissue leads to a

¹ Zeit. klin. Med., 1905 (56), 111.

² An interesting observation concerning gangrene of the lung has been made by Eijkman (Cent. f. Bakt., Abt. 1, 1903 (35), 1), who found in this condition bacteria that secrete an enzyme dissolving elastic tissue.

diffusion of water into this area, with consequent swelling, and often a lifting up of the skin in the form of blisters.

Emphysematous gangrene,¹ usually produced by gas-forming anaërobic bacteria, particularly by *B. aërogenes capsulatus*, may also possibly be produced by *B. coli communis* in diabetic patients in whose blood and tissues there may occur sufficient sugar to permit of gas-formation. Hitschmann and Lindenthal² found that the gas produced in cultures by an anaërobic organism which they isolated from a case of emphysematous gangrene, consisted of 67.55 per cent. hydrogen, 30.62 per cent. carbon dioxide, and traces of ammonia and nitrogen; this corresponds to the statement of Welch and Nuttall that the gas in the tissues of infected animals is inflammable. Dunham³ found that the gas produced by *B. aërogenes capsulatus* in cultures has the following composition: Hydrogen, 64.3 per cent.; carbon dioxide, 27.6 per cent.; other gases, probably chiefly nitrogen, 8.1 per cent.

RIGOR MORTIS⁴

This topic may be appropriately considered in connection with cell death, since it is a characteristic change occurring after general death. All forms of muscle, striped, smooth, and cardiac, undergo this change, which is shown by a shortening and thickening of the muscle, which also becomes opaque and hard. Rigor mortis begins first in the heart muscle, according to Fuchs,⁵ but it is generally observed first in the eyelids, then in the muscles of the jaw, from which point it proceeds downward, although the upper extremities may not become rigid before the lower. The time of onset is extremely variable, but the following general rules may be stated: All conditions that lead to excessive muscular metabolism, with its resulting increase in the acidity of the muscle fluids, will hasten the onset of rigor mortis; thus, people killed suddenly during violent activity may remain almost in the position in which they met death. Acute fevers, strychnine poisoning, tetanus, etc., cause likewise a rapid onset of rigor, which may, indeed, appear almost simultaneously with death, or even before the heart has stopped beating. When a healthy individual meets death with-

¹ Complete literature by Fraenkel, *Ergebnisse der Pathol.*, 1902 (8), 403; and by Welch, *Johns Hopkins Hosp. Bull.*, 1900 (11), 185.

² Quoted by Fraenkel.

³ *Johns Hopkins Hosp. Bull.*, 1897 (8), 68.

⁴ Literature, see v. Fürth, *Ergeb. der Physiol.*, Abt. 1, 1902 (1), 110; and references cited in text.

⁵ *Zeit. f. Heilk.*, 1900 (21, Path. Abt.), 1.

out previous exertion, rigor does not usually appear for four or six hours, but will be hastened by heat and retarded by cold. Death from hemorrhage or asphyxia is followed by a slow development of the rigor. Under ordinary conditions rigor usually begins between the first and second hour after death and is complete in one or two more hours.¹

The duration of rigor mortis also is influenced by many factors. In general, it may be said that the duration is in direct relation to the rapidity of onset, and also to the musculature of the individual. Therefore, in an emaciated individual dying with fever, rigor may appear and disappear again within two or three hours, or, indeed, escape observation altogether. The body of a muscular man dying from accident or hemorrhage may, on the other hand, show rigor for two or three weeks if kept in a cold place. Once the rigor has been broken by force, it does not again return.

Rigor mortis may be produced even before death, through poisons (monobromacetic acid, quinine), and its occurrence, even postmortem, does not necessarily mean that the muscle is dead, for if the part is transfused with a salt solution the rigor may be removed, and the muscle will then be found to react to stimuli. This indicates that the chemical changes of rigor mortis are not very profound.²

The chemistry of the changes involved in rigor mortis has been a much-contested problem. Two chief doctrines have been supported: one that rigor was not essentially different from ordinary muscular contraction except in degree, and perhaps due to a loss of inhibition to contraction. The other looks upon it as a coagulation similar to the coagulation of the blood; and this idea, it may be said, has had the most general acceptance. Brücke in 1842 supported this view, and in 1859 Kühne extracted from muscle a plasma which coagulated like ordinary blood plasma. The proteid which formed the clot is called *myosin*, and its coagulated antecedent, *myosinogen*.

This experiment has been since repeatedly verified and amplified, especially by v. Fürth and by Halliburton,³ who have separated more definitely the proteids concerned in coagulation, and found them to be globulins. There seem to be two: one, coagulating at 47°, called *paramyosinogen* (Halliburton), constitutes but about one-fifth of the total clotting globulin, and

¹ Rigor mortis may develop in the dead fetus while in the womb, but it generally disappears within five or six hours. Literature by Wolff, Arch. f. Gyn., 1903 (68), 549; Das, Brit. Jour. of Obstet., 1903 (4), 545.

² See Mangold, Pflüger's Arch., 1903 (96), 498.

³ "Chemistry of Muscle and Nerve," 1904.

passes readily into the insoluble clot, *myosin*; the other, which coagulates at 56° , constitutes the remaining four-fifths, is called *myosinogen* (Halliburton), or *myogen* (v. Fürth), and before becoming changed into myosin it passes through a soluble stage called *soluble myogen-fibrin*, which is coagulated at the remarkably low temperature of 40° .

By analogy with fibrin-formation we should expect this clotting also to be brought about by an enzyme, but this has not been proved. Calcium is of influence, favoring coagulation greatly, but its presence is not absolutely essential (v. Fürth). Of particular importance is the acid reaction of the dead muscle. Normal muscle is amphoteric when at rest, but when active the reaction becomes more and more acid, as it also does when the circulation is shut off, and hence it increases greatly after death. The acidity is due chiefly to lactic acid (although the neutral phosphates may become converted into acid phosphates in the presence of the lactic acid, and thus seem to contribute to the acidity), and may increase in twenty-four hours after death by from 6.7 to 12.8 c.c. of $\frac{n}{10}$ acid for each 100 grams of muscle (v. Fürth¹). The same author found that although the amount of acid might become in time sufficient to cause coagulation of the muscle proteids by itself, yet actually rigor mortis appears before the acidity has reached any such degree. We may conclude that the acidity of the muscle hastens the clotting, possibly by favoring some undemonstrated coagulating enzyme, and in late stages it may become so great as to precipitate the proteids that are not involved in the clotting. This readily explains why the time of appearance of rigor is so modified by the amount of muscle metabolism before death. It is, indeed, possible to produce rigor in living animals by transfusing a limb with slightly acid salt solution,² and in strychnine-poisoning the muscular spasm may pass imperceptibly into rigor mortis.

In all probability the disappearance of rigor mortis depends upon beginning autolysis of the clot by the intracellular proteases of the muscle, which act best in an acid medium. It is improbable that the degree of acidity ever becomes so high that the myosin is redissolved through a conversion into acid albumin (syntonin), as was formerly supposed.

¹ Hofmeister's Beitr., 1903 (3), 543.

² The hardness of a limb from which the blood-supply has been shut off by thrombosis or embolism, and also much of the cramp-like pain, is probably due to rigor mortis in the muscles caused by acid formation under conditions of sub-oxidation.

CLOUDY SWELLING¹

The characteristic appearance of organs the seat of cloudy swelling, which is frequently likened to a "scalded" appearance, suggests that the change consists in a coagulation of the cell proteids, which idea is supported by the similarity of the microscopic changes observed in the cells and the earliest microscopic changes observed in cells after heating gently to about their maximum thermal point. On the other hand, the granules in cloudy swelling are generally described as being soluble in dilute acetic acid and dilute KOH, which indicates that they are not the result of ordinary heat coagulation. If we bear in mind, however, that cloudy swelling probably does not represent one single change, it may be possible to arrive at some understanding of the chemical changes that occur in the process. Albrecht² considers, with good reason, that we may have a granular appearance of cells which is simply an exaggeration of the normal granular structure, and, although it may be observed in tissues moderately affected by toxins, or in starvation, or in transitory anemia, the change is still to be looked upon as little more than physiological in response to stimuli and overwork. Such a "cloudy swelling" may also occur in cells in the beginning of autolysis, or simply under the influence of salt solution. If the injury is greater, however, as in profound sepsis, or extreme local anemia, the granules become coarser, less soluble in acetic acid and KOH, and droplets resembling "myelin" make their appearance. If the injury is still more severe, true coagulation of the granules occurs, and they become insoluble, the fatty droplets become more prominent, and the cell reaches a condition that may with propriety be termed necrosis or fatty degeneration, or both. There is no very sharp line separating necrosis and cloudy swelling, especially if we consider only the changes in the cytoplasm. In the earliest stages the granules are perhaps due, in some cases, to simple aggregation of the colloids, without the development of a true coagulation, and so the granules are still soluble. Possibly bacterial toxins may also cause soluble precipitates, but this does not appear to have been established. Halliburton has shown that temperatures that may be reached in high fevers can cause turbidity in solutions of cell proteids, and hence heat precipitation may be partly responsible for the turbidity of cells in cloudy swelling, but it is doubtful if the granules thus formed would be soluble in acetic acid.

¹ Review of general features by Landsteiner, Ziegler's Beitr., 1903 (33), 237.

² Verh. Deut. Path. Gesell., 1903 (6), 63.

We may speak with more assurance concerning the swelling of the cell, and attribute it to an increase in the osmotic pressure of the cell contents, with consequent taking up of water. The rise in osmotic pressure is probably due to abnormally rapid splitting of proteids with incomplete oxidation of the substances formed, which results in formation of many crystalloid molecules with high total osmotic pressure, from a smaller number of colloid molecules with almost no osmotic pressure. It has frequently been shown that the cell-walls do not lose their semipermeable character until the death of the cell occurs; hence in cloudy swelling water diffuses in much more rapidly than the crystalloids can diffuse out,¹ causing a hydropic swelling. This hypothesis is supported by the observations of Cesaris Demel,² who found that by modifying the osmotic conditions of the cells, particularly epithelial cells, he could closely reproduce many of the characteristic features of parenchymatous degeneration. It is possible, also, that too high concentration of crystalloids within the cells may be a factor in the precipitation of the cell colloids. In view of the fact that in the earliest stages of autolysis histologic and microscopic changes closely resembling those of cloudy swelling are pronounced, and that organs the seat of cloudy swelling notoriously undergo autolysis with extreme rapidity after death, we may also consider that this process is possibly in part responsible for the change of ordinary *intra vitam* cloudy swelling. The appearance of fine granules of lipid substance (myelin or "protagon" (?)) in cells during autolysis and during cloudy swelling is cited by Orgler³ in support of this idea, and he found by chemical analysis of organs showing cloudy swelling that there is definite evidence of autolytic decomposition of the proteids and an increase in the water content.⁴ Landsteiner, through his studies of cloudy swelling in human material also came to the conclusion that autolysis is an important element in its production.

"Waxy" degeneration of muscles, although usually resulting from the action of toxic substances, is entirely different from cloudy swelling, in that the cytoplasm becomes homogeneous and not granular. Dr. A. P. Mathews has suggested to me, as a possible explanation, that the change is allied to the action of acids upon fibrin, which causes the fibrin to swell up and become homogeneous. As we know that abundant acid

¹ See introductory chapter concerning osmosis; also discussion of edema.

² Lo Sperimentale, 1905; Cent. f. Path., 1905 (16), 613.

³ Virchow's Arch., 1904 (176), 413.

⁴ Verh. Deut. Path. Gesell., 1903 (6), 76.

formation goes on in muscle-cells under pathological conditions, this explanation seems to have considerable value. The results of some preliminary experiments that I have performed support this hypothesis.¹

Summary.—Putting all these facts together, we may look upon the term cloudy swelling as applying to many different sorts of processes which may be caused by many different factors, the common features being the precipitation or the coagulation of part of the dissolved cell proteids (often with the separation of the intracellular fat from the proteids, so that it becomes microscopically visible) and the imbibition of water.

“Hydropic degeneration” may be properly considered as differing from cloudy swelling chiefly in the excessive prominence of the absorption of water.

¹ Muscles showing the *reaction of degeneration* have been analyzed by Rumpf and Schumm (Deut. Zeit. f. Nervenheilk., 1901 (20), 445), who found a great increase in the fatty matter, which was about fifteen times the normal amount. The muscle, deducting the fat, showed a loss of solid matter and an increase of water; sodium and calcium were increased, potassium decreased.

CHAPTER XIV

RETROGRESSIVE PROCESSES (CONTINUED)

Fatty, Amyloid, Hyaline, Colloid, and Glycogenic Infiltration and Degeneration

FATTY METAMORPHOSIS

IN 1847, in the first number of his *Archiv*, Virchow divided the forms of fatty changes that may occur in pathological conditions into two groups—"infiltration" and "degeneration"—a division that has since become classical. By infiltration he indicated the excessive accumulation of fat in the cells in the form of large droplets, without destruction of the nucleus or irreparable damage to the cells, and by the use of the term infiltration he implied his belief that the fat entered the cell from without. When the fat remained in the form of fine droplets and the cell became much disintegrated, Virchow considered that the fat was derived from the breaking down of the cell proteids, and hence the process was considered to be a fatty degeneration of the protoplasm. Since that time scarcely any other subject in pathology has been more warmly discussed than that of the origin of the fat in fatty degeneration, and an appalling amount of literature has accumulated concerning the question involved. It will be impossible to give more than the essential facts that have been developed, referring the reader for the full details of the discussion and evidence to the numerous compilations of literature, particularly those of Rosenfeld,¹ and to the original articles cited in the text.

PHYSIOLOGICAL FORMATION OF FAT

Concerning the normal formation of fat we may summarize the evidence as follows :

- (1) A large proportion of the fat of the body comes from

¹ "Fat Formation," *Ergebnisse der Physiol.*, Abt. 1, 1902 (1), 651; *ibid.*, 1903 (2), 50. Also see discussion in the *Verh. Deut. Path. Gesell.*, 1904 (6), 37-108, and the review by Leathes in his "Problems in Animal Metabolism," 1906, pp. 71-121. Concerning modern theories of rôle of lipase in fat metabolism see Chap. iii. Other reviews of literature on pathological fat formation by Christian, *Johns Hopkins Hosp. Bull.*, 1905 (16), 1; Herxheimer, *Ergebnisse der Pathol.*, 1902 (8), 625; Löhlein, *Virchow's Arch.*, 1905 (180), 1; Pratt, *Johns Hopkins Hosp. Bull.*, 1904 (15), 301 (particular reference to heart). Later references of importance cited in the text.

the fat taken in the food, as also does the fat of the milk. This can be shown, as Rosenfeld particularly demonstrated, by starving an animal until it is as free from fat as possible, then feeding with a large amount of some fat that is of a type different from that normally found in the animal; the new fat that is then laid up in the fat depots of the animal will partake of the characters of the fat given in the food. In case the animal is lactating, the milk-fat will also resemble the fat of the food.¹ As a matter of fact, the body fat is not of constant composition, even in the same individual; it varies greatly with age, having much less olein in infancy than in later years, varying somewhat in composition in the different fat depots in the same body, and apparently being more or less modified by diet.

(2) Fat may also be formed from carbohydrates. According to Rosenfeld, this fat differs from the fat formed on mixed diet in having less olein in proportion to the palmitin and stearin, and it is deposited particularly in the subcutaneous and mesenteric tissues rather than in the liver. Man does not seem to form fat readily from carbohydrates, but rather burns them to protect his proteids; on the other hand, swine and geese readily form fat from carbohydrates. As the fatty acid radicals of ordinary fat ($C_{18}H_{36}O_2$, $C_{16}H_{32}O_2$, $C_{18}H_{34}O_2$) are much larger than the carbohydrate radicals, a process of synthesis must be involved in the formation of fat from carbohydrates.²

(3) Proteids are a possible source of fat, but it has not been established that they are either a common or an important source of fat in either physiological or pathological conditions, or, indeed, that they really ever do form fat. Upon this statement rests our present tendency to refute the long-cherished conception of fatty degeneration as a true degeneration of cell proteids into fat, as suggested by Virchow. This view was supported by the earlier work of Voit and his school, who believed that they had demonstrated that animals could form fat from proteid food, and their work was for a long time accepted as correct. Later Pflüger and his pupils pointed out what seem to have been essential errors in these investigations, and, after much discussion and experimentation, the majority

¹ See Engel, *Zeit. physiol. Chem.*, 1905 (44), 353. Thiemich (*Jahrb. f. Kinderheilk.*, 1905 (61), 174) has also found evidence that the fat of the fetus is transported from the fat depots of the mother.

² This, Magnus-Levy suggests, may be accomplished through lactic acid which is formed from sugar, and then, after reduction to an aldehyde, several of these molecules are combined into the higher fatty acid. See Leathes, *loc. cit.*, p. 82.

of physiologists now support the view advanced in the sentence opening this paragraph. Since proteids contain carbohydrate groups, and since fats can be formed from carbohydrates, the possibility of the formation of fats from the proteids in this indirect way cannot be denied. It is also possible that the nitrogen-containing groups may be split out of the amino-acids of the proteid molecule, and that the non-nitrogenous residues can then be built up into fatty acid molecules as large as the molecules of stearic, palmitic, and oleic acids; but we have no proof that either of these processes occurs in the normal cell or in the cell that is undergoing degeneration.

PATHOLOGICAL FAT ACCUMULATION

For a long time fatty degeneration was looked upon as one of the chief evidences that fat was formed directly from proteid, for the cell protoplasm seemed, morphologically, to be changed directly into fat in this process. Additional support was also claimed from the supposed increase in fat in the ripening of cheese; from the formation of abundant fat by maggots living in fat-poor blood or fibrin; and by the apparent conversion of proteids into fatty acids and soaps in the postmortem change, *adipocere*. But it has now been well established that there is no true conversion of proteid into fat in the fatty degeneration produced experimentally by poisoning with phosphorus, etc.,¹ and the other supposed instances of fat-formation above cited have been discredited by various methods which it will not serve our purpose to discuss here, beyond mentioning that one of the chief sources of error lies in the fact that many fungi and bacteria² can form fat from proteid.

It having been rendered probable that fat was not formed by disintegration of the proteid of the degenerating cells, it remained to determine what the source of the fat observed in the cells under pathological conditions might be, and this part of the problem has been largely cleared up by Rosenfeld. This investigator proceeded as follows: Animals were starved until they were extremely poor in fat, then fed upon easily identified foreign fats, such as mutton tallow (which has a high melting-point and can combine with little iodine) or linseed oil (which has a low melting-point and can combine with much iodine). The animals under these conditions laid up in their fat depots, including the liver as well as the subcutaneous tissues, large

¹ See Taylor, Jour. Exp. Med., 1899 (4), 399.

² See Beebe and Buxton, Amer. Jour. of Physiol., 1905 (12), 466; Slosse, Arch. Internat. Physiol., 1904 (1), 348.

quantities of these foreign fats. By starving again for a few days the foreign fat was removed from the liver, leaving still a large amount in the other storehouses, and the animals were then poisoned with phosphorus or other poisons that cause a typical fatty degeneration of the liver and other viscera. When the fat was extracted from the fatty liver of these animals, it was found that the new fat that had appeared in the liver during the process was not normal dog fat (which it should have been if formed by degeneration of the cell proteids), but was, in part, of the same type as the foreign fat which the animals had deposited in their subcutaneous tissues and other fat storehouses. Furthermore, it was found that animals starved to an extremely low fat content do not develop the typical fatty liver of phosphorus-poisoning, a fact which Lebedeff had already noted in a case of phosphorus-poisoning in an emaciated patient. Therefore, it seemed evident that *the fat accumulating in the liver during fatty degeneration is not derived, as Virchow thought, through a transformation of cell proteids into fat, but rather is an infiltrated fat brought in the blood from the fat deposits of the body to the disintegrating organ.* This work has since been corroborated and extended by many observers, and its correctness can now hardly be questioned.¹ "Fatty degeneration," therefore, differs from "fatty infiltration" chiefly in the fact that in the former the process is associated with serious injury to the cell, caused by the action of toxins or loss of nutrition, while in the latter the cell is not seriously injured and is capable of returning to its normal condition whenever the fat is removed.²

Fatty "Degeneration" without Infiltration.—By showing that the new fat in fatty livers is infiltrated fat, Rosenfeld did not entirely clear up the subject, for, in the course of his analyses of organs that were macro- or microscopically the seat of fatty degeneration, he found that there is not always any correspondence between the amount of fat that seems to be present, as determined by microscopic methods, and the amount that chemical analysis shows to be present. This

¹ Schwalbe (Verh. der Deut. Path. Gesell., 1903 (6), 71) claims that in a similar way iodine compounds of fat can be demonstrated to be transported into the fatty organs. His analyses were merely qualitative, and by quantitative determinations I was unable to corroborate his results (Zeit. f. physiol. Chem., 1905 (45), 412).

² A striking proof of the lack of injury associated with fatty infiltration is shown by the fatty infiltration frequently seen in the liver, especially of alcoholics, in which it may be difficult to find, microscopically, any cell cytoplasm because of the fat, the tissue looking like fatty areolar tissue; and yet there may be no clinical evidence whatever that the liver function has been impaired by the process.

is particularly true of the kidney. Thus, the amount of fat present in normal kidneys (dog) was found to vary between 18.5 per cent. and 29.12 per cent. of the dry weight, the average being 21.8 per cent.; whereas, after producing a typical "fatty degeneration" by means of phosphorus and other poisons, the fat content was still found to be between 16.9 per cent. and 22.6 per cent.¹ In all instances the amount of fat in kidneys showing typical fatty degeneration under the microscope was found equal to or less than the normal amount—it was never increased. The same conditions were found to obtain in human kidneys that showed fatty metamorphosis. Microscopic examination of specimens stained with the specific fat stains,² therefore, gives no indication of the amount of fat contained in a degenerated kidney. A pathologic kidney containing 16 per cent. of fat (18 per cent. is about the average amount of fat in normal human kidneys) may show extreme "fatty degeneration" under the microscope, whereas another kidney may contain as much as 23 per cent. of fat, yet not show any fat whatever by staining methods.

The explanation of this remarkable discrepancy is as follows: Every tissue and organ seems to contain a greater or less amount of fat, varying from 5 per cent. to 20 per cent. of the total dry weight of the organ in the case of most of the important tissues, yet this fat is usually held in such a form that it cannot be stained by any stains available for the purpose. Thus in the kidneys, as before remarked, we may have as much as 23 per cent. of fat present and yet be entirely unable to stain any of it. The greater part of this fat seems to be essential to the cell, for it cannot be removed by the most extreme starvation; *e. g.*, the liver of the most emaciated dogs may contain 10 per cent. to 20 per cent. of fatty substances. Furthermore, the same resistance

¹ Concerning the normal intracellular fats see introductory chapter.

² Fat-staining involves several principles of interest in this connection. Osmic acid (OsO_4), the longest used for this purpose, is reduced to OsO_2 by oleic acid, imparting a black or dark-brown color to the fat; but it does not stain saturated fatty acids, such as palmitic or stearic acid. Thus, Christian found in pneumonic exudates fat that stained by other methods but not by osmic acid, apparently because it contained no oleic acid (*Jour. Med. Research*, 1903 (10), 109). Sudan III and scarlet R (*fat ponceau*) are two synthetic dyes which stain fat in a purely physical way, entering and remaining in the fat-droplets because they are much more soluble in fat than they are in water or alcohol. (Fully discussed by Michaelis (who introduced scarlet R) in Virchow's Arch., 1901 (164), 263; and by Mann, "Physiological Histology," p. 306.) These stains have the advantage of staining all sorts of fats and not staining other substances that may reduce osmic acid. Fatty acids and soaps may be stained with copper acetate, which forms a green copper salt, and thus be distinguished from fats (Benda, Virchow's Arch., 1900 (161), 194).

is shown by part of the fat to extraction with ether. A certain proportion of the fat can be extracted readily in twenty-four hours or less by ether, but after this time no more can be made to leave the tissues. Apparently the rest of the fat is held in a combination (which seems to be chemical rather than physical) that is insoluble in ether. By digesting the tissue for a short time by pepsin, however, the rest of the fat becomes freed (suggesting that it is the proteids with which it is combined), so that it can then be readily dissolved out in ether.¹ We see, therefore, that much of the fat of normal cells is so firmly combined that it cannot be dissolved in ether, and under normal conditions all, or nearly all, of it cannot be stained. (This applies particularly to the parenchymatous organs; the fat of the areolar tissue is all readily extracted—Taylor.) But when pathological changes in the cells result in decomposition of the cell proteid through autolysis, part of this normally invisible fat is set free, and, becoming visible, produces the so-called "fatty degeneration." This explains the observations of Rosenfeld, cited above, that kidneys may show much fat to the naked eye and microscopically, when they actually contain even less than normal amounts of fat. Taylor² advanced this explanation, and supported it experimentally by showing that during fatty degeneration this protected fat actually is liberated, some two-thirds becoming ether-soluble in an experiment performed with phosphorus-poisoned frogs. As further support may be mentioned the fact that organs undergoing experimental autolysis show microscopically an apparently typical fatty degeneration, although analyses show that no actual increase in fat occurs.³

Relation of Anatomical to Chemical Changes.—

From the facts brought out in these various experiments we must consider that the anatomically established condition of "fatty degeneration" represents either or both of two conditions: (1) It may result from an increase in the normal quantity of fat in an organ undergoing parenchymatous degeneration, through an infiltration of fat from the outside; this is particularly true of the fatty degeneration of the liver; (2) or there may be no increase in the total amount of fat, but the

¹ Chloroform will separate this fixed fat from the tissues; and alcohol-hardened tissues hold much less of the fixed fat than do dried tissues.

² Jour. Med. Research, 1903 (9), 59.

³ Kraus, Arch. exp. Path. u. Pharm., 1886 (22), 174; Siegert, Hofmeister's Beitr., 1901 (1), 114. Waldvogel (Virchow's Arch., 1904 (177), 1), however, claims that the "protagon" and "jecorin" increase in autolyzing organs, while the lecithin decreases, and believes that proteids may indirectly give rise to fat. There are numerous questionable features concerning these results, and they cannot be considered as final.

invisible fat becomes visible through autolysis of the cell proteids. (3) Finally, of course, both factors may occur together. Of these various forms, in only the first and last can we properly consider the organ "fatty," and the form that will occur seems not to depend upon the cause of the cell injury, but rather upon the organ under consideration. In a study of the relation of the morphological to the chemical changes Rosenfeld¹ arrived at the following results:

Normal human hearts contain, on an average, 15.4 per cent. of fat; the hearts showing fatty degeneration contain 20.7 per cent., on an average. The pancreas, which normally contains 15.8–17.4 per cent. of fat, also contains an increased amount of fat when showing fatty degeneration. The liver, however, takes on by far the greatest amount of fat after "steatogenetic" poisons, and the microscopic picture gives a very good approximation of the amount of fat it contains.² Apparently in these organs any excessive fat above the normal is observable microscopically, although the normal fat content is not, and only in these three organs could Rosenfeld find an actual increase in fat after poisoning with phosphorus, etc. It would seem, on the other hand, that there is not often a real increase in the fat content of the "fatty" kidney.³ Normal spleen contains 14.2 per cent. of fat, and lung 17.3 per cent., but in both, "fatty degeneration" results in a lowering of this quantity. Degenerations in the nervous tissue, which Virchow considered the best evidence of the conversion of protoplasm into fat, also show a marked *decrease* in fat, and voluntary muscle shows no increase in the normal quantity after poisoning. In general, these experiments support the contention of Taylor concerning the disclosure of the invisible fat through autolysis.⁴

¹ Berl. klin. Woch., 1904 (41), 587.

² In fatty livers in phosphorus-poisoning the amount of fat may reach 75 per cent. of the dry weight. Accompanying the fat increase are increase in water and a relative or absolute decrease in proteids, probably due to cell autolysis. In acute yellow atrophy a similar decrease in proteid occurs, but without an increase in fat. (See v. Starck, Deut. Arch. klin. Med., 1884 (35), 481.)

³ This is contradicted by Landsteiner and Mucha (Cent. f. Path., 1904 (15), 752) and by Löhlein (Virchow's Arch., 1905 (180), 1) and Rosenthal (Deut. Arch. klin. Med., 1903 (78), 94), but is supported by Orgler (*ibid.*, 1904 (176), 413). See also the recent studies by Rosenfeld on the effects of various steatogenic poisons on different organs, in Arch. f. Exp. Path. u. Pharm., 1906 (114), 179 and 344. It is probable that the truth lies between the opposing views, namely, the kidney may under some conditions take up fat from the blood, but it does so to a much less extent than the liver, and it may sometimes show marked fatty change anatomically without corresponding increase chemically.

⁴ Pieces of tissue implanted into animals may show a peripheral fatty metamorphosis or infiltration, yet show upon analysis a decreased fat content (Dietrich, Verh. Deut. Path. Gesellsch., 1905 (9), 212).

Lecithin and Other Intracellular Lipoids.—It has often been suggested that the lecithin of the cell might act as a source of the fat in fatty degeneration, but it has been quite conclusively shown that this is not the case, numerous investigators having found that the amount of lecithin remains nearly normal in cells even during the most extreme fatty degeneration.¹ The lecithin may be, and undoubtedly is, one of the fatty substances that become visible during cell autolysis, and presumably other lipoids also appear.

Kaiserling and Orgler² have described under the non-committal name of "*myelin*" certain intracellular droplets that may be found in the cortical cells of the normal adrenal, in amyloid kidneys, pneumonic exudates, tumor cells, retrogressive thymus tissue, corpus luteum, and bronchial secretions; and which differ from fat in being doubly refractile and in staining but faintly gray with osmic acid, although taking up fat stains well. Their average size is 4–6 microns, and they dissolve in ether and chloroform readily, but poorly in alcohol. Probably this myelin is one of the cell lipoids, possibly "*protagon*" made visible by cell degeneration, for except in the adrenal the cells containing it are in a necrobiotic state. This is supported by Albrecht's observation that post-mortem myelin formation is checked by heating the cells to 58°–62°, a temperature which destroys the autolytic enzymes.³

Summary.—We must conclude, therefore, that fatty degeneration of an organ means, in the case of the liver, myocardium, and pancreas an infiltration of fat from outside into cells which have been degenerated by the action of poisons or other injurious influences. In the kidney, spleen, and muscles an increase of fat seldom occurs from these causes, but the cells may show a marked fatty metamorphosis through the setting free of the invisible intracellular fat by autolytic changes.

CAUSES OF FATTY METAMORPHOSIS

Nevertheless, the old anatomical distinction of infiltration and degeneration still remains, provided we do not hold to the original idea that the term degeneration implies that the cell proteid has been converted into fat; for we must recognize that under some conditions the cells may take up great quantities of fat without suffering any appreciable degenerative changes, whereas in other instances the appearance of fat is associated

¹ Lusena, *Lo Sperim.*, 1903 (57), 29; Rubow, *Dissert.*, Copenhagen, 1903; Rubow, *Arch. f. exp. Pathol.*, 1905 (52), 173. Waldvogel, however, maintains that in fatty degeneration and in autolysis there occurs a decrease in the lecithin, associated with an increase in "*jecorin*," "*protagon*," fatty acids, neutral fats, and cholesterin. There is so much doubt concerning the chemical status of "*jecorin*" and "*protagon*" that these statements are in need of much confirmation. (See Waldvogel and Mette, *Münch. med. Woch.*, 1906 (53), 402, for review of Waldvogel's work.)

² *Virchow's Arch.*, 1902 (167), 296.

³ *Cent. f. Path.*, 1904 (15), 982. See also Orgler, *Virchow's Arch.*, 1904 (176), 413; and Albrecht, *Verh. Deut. Path. Gesellsch.*, 1903 (6), 95.

with marked and complete disintegration of both nucleus and cytoplasm. Furthermore, we have yet to explain why, under some conditions, the fat is removed from the fat depots to be stored up in the liver or other organs. By applying the facts recently brought out concerning fat metabolism, particularly by Kastle and Loevenhart,¹ a satisfactory explanation seems to be possible. Fat is always utilized and transported in the form of its two constituents, fatty acid (or soaps) and glycerin, which are diffusible and soluble. It enters and leaves the cells in this condition, being split or combined, as may be necessary to produce equilibrium, by the action of lipase, which is present within the cells and in the blood and lymph. Under normal conditions there is little free visible fat in the cells of the parenchymatous organs, because it is largely used up through oxidation of the glycerin and fatty acids by the action of the intracellular oxidases. Where there is abundant lipase and but little oxidative activity, as is the case in the areolar fat tissue, fat accumulates in large amounts. When, for any reason, the oxidative power of the parenchymatous organs is reduced, fat accumulates in them as it does in the fat depots normally, and we have an excess of fat in the parenchymatous cells; thus, in pulmonary tuberculosis, severe or protracted anemias, etc., a great accumulation of fat occurs, particularly in the liver, where normally active oxidative processes continually balance the action of the abundant lipase of the liver-cells.

If the fat accumulates in cells that are structurally normal or nearly so, the fat-droplets fuse together under the pressure of the cytoplasm, and we get the picture of a typical fatty infiltration. If the cells are much disintegrated through the action of the poison,—*e. g.*, phosphorus, bacterial toxins, etc.,—the accumulating fat-droplets are not crowded into one large droplet, but lie free in the granular débris of the disintegrating cell, constituting the typical appearance of fatty degeneration. Fatty degeneration is usually brought about by poisons, while fatty infiltration depends usually upon decreased oxidation, due to lack of either oxygen or hemoglobin in the blood. If the anemia is extreme, however, the cells degenerate, and then we find a true fatty degeneration caused by lack of oxygen. Thus, in an anemic infarct fat accumulates about the periphery of the dead area,² probably because fatty acids and glycerin diffuse in slowly from the surrounding parts where circulation still goes on, and are built up into fat by the cell lipase, for in anemic

¹ See consideration of this topic on page 67.

² Fischler, *Cent. f. Path.*, 1902 (13), 417.

areas the intracellular oxidases cannot destroy these substances as they normally do, because of lack of oxygen. The accumulation of fat in dead areas depends, therefore, on the fact that the constituents of fat can diffuse into the dead tissue, whereas the oxygen, being held in the corpuscles, cannot enter the anemic area.

It is to be supposed that poisons also cause fatty degeneration in a similar way—by interfering with oxidation. We have much evidence that in phosphorus, chloroform, and other poisoning associated with fatty degeneration of the liver, oxidation is impaired.¹ If we imagine for a moment, a cell in which oxidation is checked by any means, we shall have in this cell the lipase and the proteolytic enzymes not balanced, as they normally are by the action of the oxidases, and hence the processes of cell autolysis and of the accumulation of fat by the lipase will go on uncontrolled. The result will be a disintegrated cell containing many fat-droplets, *i. e.*, fatty degeneration.²

Summary.—Fatty metamorphosis involves changes of two kinds. First, infiltration of fat, which occurs when the oxidative power of the cells is decreased, so that fat is not destroyed, but is accumulated from the blood under the influence of the lipase of the cells; if there is not any serious injury to the cells, the histological changes consist in the accumulation of one or a few large droplets of fat in each cell, constituting the condition known anatomically as “fatty infiltration.” This occurs, pathologically, chiefly in the liver. If at the same time the cytoplasm is disintegrated through autolytic changes, the fat-droplets do not fuse, but remain as small, more or less discrete, fat granules among the granules of cell debris, constituting the microscopic picture of “fatty degeneration”; this condition occurs particularly in the heart and liver.

Second, each cell contains a large amount of fat (5–25 per cent. of its dry weight), which is so combined that it cannot be detected microscopically; this fat may be liberated during the autolytic processes of cell disintegration and become visible,

¹ See Welsch, *Arch. int. de pharm. et therap.*, 1905 (14), 211.

² Interference with oxidation does not necessarily imply destruction of the oxidases. As yet we know practically nothing concerning the oxidases of the cells in disease, and the above hypothesis has yet to be demonstrated. Ducheschi and Almagia (*Arch. Ital. Biol.*, 1903 (39), 29) found the normal amount of lipase in phosphorus-livers, but also observed no decrease in ability to oxidize salicylic aldehyde, which, however, does not prove a normal power to oxidize fats. Gierke's observation (*Ziegler's Beitr.*, 1905 (37), 502) that glycogen and fat accumulate under identical conditions might be cited as indicating decreased oxidative power, were it not in direct contradiction to the results obtained by Rosenfeld.

constituting a macroscopical and microscopical degeneration, but without any actual increase in fat—this condition occurs particularly in the kidney and nervous system. Third, a combination of both of the above processes, infiltration of fat and liberation of masked intracellular fat, may occur simultaneously in an organ.¹

PROCESSES RELATED TO FATTY METAMORPHOSIS

ADIPOCERE

This apparent transformation of the substance of dead bodies into a wax-like material was for a long time looked upon as evidence of a transformation of proteid into fat, but in the light of more recent investigations this view can hardly be held. Adipocere is the product of a process that occurs particularly in bodies buried in very wet places or lying in water, and results in an apparent replacement of the muscles and other soft parts (but not the glandular organs) by a mass consisting of a mixture of fatty acids in crystalline and amorphous form, and soaps, particularly ammonium, magnesium, and calcium salts of palmitic and stearic acid (the oleic acid largely disappearing during the process). The resulting material is absolutely resistant to putrefaction, and hence remains intact for many years. This replacement of the soft parts is, however, only apparent, for the total weight of a body in this condition is much lighter than that of the original body; indeed, one is always surprised at the light weight on lifting such a specimen. Adipocere occurs almost exclusively in fat bodies, and it seems probable that all the soaps and fatty acids found are *formed from the original fats of the corpse*. These gradually flow into the places left by the disintegrating muscle, etc., a process that occurs readily in cadavers, according to Zillner;² or the infiltration may be accomplished through diffusion of the ammonium soaps formed during the decomposition. As the subcutaneous fat is hardened by the formation of soaps, and the bones remain to hold the parts in position, the general form of the body is preserved, creating the impression that its entire substance has been converted into adipocere, when the total mass may actually weigh but twenty

¹ The above conception of the processes involved in fatty metamorphosis has been held by the writer for several years, and is more fully discussed in other publications (Jour. Amer. Med. Assoc., 1902 (38), 220; *ibid.*, 1906 (46), 341). Ribbert (Deut. med. Woch., 1903 (29), 793) has also advanced a similar explanation for the morphological differences between fatty "degeneration" and "infiltration," *i. e.*, that the degenerative changes are independent of the fatty accumulation.

² Vierteljahrsh. f. gericht. Med., 1885 (42), 1.

pounds or so, and, according to Zillner's estimate, not more than one-tenth of the muscle substance is replaced by adipocere. This false impression is probably responsible for much of the mistaken idea concerning the conversion of muscle proteids into fatty acids.

Numerous attempts have been made to prove that muscle could be thus converted into fatty acids and soaps, but although success has been claimed by a few, the results are not entirely convincing.¹ Bacteria can convert proteids into fats, beyond a doubt, and they may do so to some slight extent in adipocere formation, but probably this factor is not important.

In the light of our present conception of fat metabolism it is probable that the process of adipocere formation occurs as follows: The fatty acids of the fat tissue are combined by the ammonia formed during putrefaction, removing these fatty acids from the normal balance of fat and fatty acids in the fat tissue; as a result, the lipase of the fat tissue continues to split up the fat, and more fatty acids are produced, which likewise go to form soaps. This continues until practically all the neutral fat has been decomposed, the glycerin diffusing rapidly away. The soluble soaps, which the bacteria do not attack, diffuse into the softened muscle tissue, which they gradually replace in part. In the meantime, from the more soluble ammonium soaps, calcium and magnesium soaps are being slowly formed, according to the usual rule of double decomposition (that the least soluble salt will be formed under such conditions). The oleic acid seems to be converted into the higher fatty acids (Salkowski).² It is also possible that the saponification is due to the gradual action of the alkaline fluids produced in decomposition of the tissues, or to the alkalinity of the water in which the body lies. Possibly bacteria may be responsible for this decomposition of the fats rather than the body lipase, for Eijkman³ has observed that certain bacteria growing in fat-containing agar produce calcium, ammonium, and sodium soaps, simulating adipocere.

Zillner⁴ gives the following scheme of the changes that take place in a cadaver undergoing adipocere formation: (1) Migration of fluid contents of the body (imbibition of blood and transudation)—one to four weeks. (2) Decomposition of superficial epidermis, then of corium—first two months. (3) Decomposition of muscle and gland parenchyma, until only the

¹ See Rosenfeld, *Ergeb. der. Physiol.*, Abt. 1, 1902 (1), 659.

² *Festschr. f. Virchow*, 1891, p. 23.

³ *Cent. f. Bakt.*, 1901 (29), 847.

⁴ *Loc. cit.*

inorganic part of the bones and the connective and elastic tissues remain—three to twelve months. (4) Migration of neutral fat, crystallization and partial saponification of the higher fatty acids in the panniculus; transformation of the blood pigment into crystalline form—four to twelve or more months.

LIPEMIA

Normally the blood contains a considerable amount of fat, varying somewhat, but not greatly, with the diet. Engelhardt¹ found in eight healthy persons an average of 0.194 per cent., with a maximum of 0.237 per cent., and a minimum of 0.101 per cent.; in five cachectic patients the quantities were quite the same. Rumpf,² in a large number of morbid conditions, however, found the solid substance of the blood to contain an average of 0.452 per cent. of fat, but with variations between 0.035 per cent. and 2.20 per cent. It is, therefore, extremely difficult to say just when the amount of fat in the blood is large enough to be considered as a lipemia. B. Fischer³ states that we may speak of a pathological lipemia when we have a distinctly cloudy blood or serum, which is clarified by shaking with ether, through the dissolving out of fat which can then be separated from the ether. Earlier writers described, incorrectly, lipemia in many conditions, but recent writers mention it chiefly as occurring in alcoholism and diabetes. By far the greatest amounts of fat are observed in the latter condition. Neisser and Derlin⁴ found 19.7 per cent. of fat in the blood of a patient with diabetic coma (after death 24.4 per cent. was found) whose urine contained 0.8 per cent. of fat, and through analysis of this and other material came to the conclusion that the fat comes directly from the chyle; *i. e.*, that it is food fat, not body fat. Fischer found an average of 18.129 per cent. in his case, including at least 0.478 per cent. of cholesterin, with no lipuria and very small amounts of fatty acids; of the fat, about 67.5 per cent. was olein.

It is an important question whether, with such high quantities of fat in the blood, fat embolism may result, for it is possible that at least some of the cases of diabetic coma are due to such fat embolism in the cerebral vessels. Ebstein⁵ considers this a possible, but not a common, occurrence, because the drop-lets are too small to cause occlusion of the vessels unless they

¹ Deut. Arch. klin. Med., 1901 (70), 182.

² Virchow's Arch., 1903 (174), 163.

³ Virchow's Arch., 1903 (172), 30. Résumé and complete literature.

⁴ Zeit. klin. Med., 1904 (51), 428.

⁵ Virchow's Arch., 1899 (155), 571.

combine to form large droplets. Fischer doubts if the droplets ever fuse together enough to cause embolism, supporting his contention both by experiments and clinical records.

The cause of lipemia has not yet been satisfactorily determined. In alcoholism it is commonly ascribed to a failure to burn fat, because of the presence of the more readily oxidized alcohol, and the common coexistence of diabetes and lipemia suggests for both a common cause; *i. e.*, lack of oxidation of fat and sugar. In corroboration may be cited the occurrence of lipemia in other conditions associated with defective oxidation; *i. e.*, pneumonia, anemia, phosphorus-poisoning. As we are still unfamiliar with the essential factors and steps in the oxidation of fat, it would be mere speculation to attempt to explain further the reason for the failure of destruction of the fat. The origin of the fat in lipemia is likewise undetermined. Ebstein considers that it arises partly from the food, partly from fatty degeneration of the cells of the blood, the vessel-walls, and the viscera. Neisser and Derlin consider it as merely food fat coming from the chyle and accumulated in the blood. Fischer believes that it is largely derived from the fat depots, and that because of loss of the lipolytic power of the blood it cannot be rendered diffusible, and hence it cannot enter the tissues where it is normally consumed.

PATHOLOGICAL OCCURRENCE OF FATTY ACIDS

Fatty acids occasionally occur free in pathological processes. The best example of this is fat necrosis (*q. v.*), where crystals of fatty acids appear in the necrotic fat-cells, arising through splitting of fat, and later becoming combined with calcium from the blood. Similar crystals, consisting of a mixture of palmitic and stearic acids, frequently called *margarin* or *margaric acid* crystals, may be found in decomposed pus, in sputum from bronchiectatic cavities, and from gangrene of the lungs, in gangrenous tissue, and in atheromatous areas. According to Schwartz and Kayser,¹ the free fatty acids, at least in pulmonary gangrene, arise from lipolysis by bacterial action rather than by the lipase of the tissues. Eichhorst found crystals of fatty acids in the neighborhood of acute patches of sclerosis in the central nervous system in *multiple sclerosis*, and McCarthy² found them in a spinal cord undergoing secondary degeneration from compression.

¹ Zeit. klin. Med., 1905 (56), 111.

² Univ. of Penn. Med. Bull., 1903 (16), 141.

The fatty acids may be stained green by copper acetate, according to Benda's method, and if then treated with hematoxylin, they turn black.¹ Fischler and Gross² state that fatty acids are present in atheromatous areas and about the margin of anemic infarcts, but are not recognizable by this method in such fatty degenerations as pneumonic exudates, caseation, etc. Klotz³ considers that calcium soaps are formed as the first step in pathological calcification, according to microchemical evidence; but a chemical investigation of the same question did not give the writer positive results.⁴

PATHOLOGICAL OCCURRENCE OF CHOLESTERIN⁵

Cholesterin in crystals is found under somewhat the same conditions as the fatty acids, and although cholesterin is not a fat, but an alcohol, its physical properties are so similar that it may be considered in this place. (See "Gall-stones," Chap. xv, for further discussion.) The characteristic large flat plates of cholesterin may be found in any tissue in which cells are undergoing slow destruction, and where absorption is poor. Therefore, they are found frequently in atheromatous patches in the blood-vessels, encapsulated caseous areas, old infarcts and hematomas, inspissated pus-collections, dermoid cysts, hydrocele fluids, etc.; especially large amounts occur in the cholesteatomatous tumors of the ear and cranial cavity.⁶ In liquids the crystals form glistening scales; in fresh tissues they may be recognized by their solubility in ether, chloroform, hot alcohol, etc., and by their color reactions. In histological specimens prepared by the usual methods the cholesterin is dissolved out, but the resulting clear-cut clefts are quite characteristic. In fresh specimens in which cholesterin crystals are present, on treatment with five parts concentrated sulphuric acid and one of water, the edges of the crystals become carmine red, then violet. Concentrated sulphuric acid plus a trace of iodine colors the crystals in sequence, violet, blue, green, and red. Hirschsohn⁷ recommends a reaction with a 90 per cent. solution of trichloroacetic acid in HCl, which gives red, then violet, then blue.

Since all, or nearly all, cells contain cholesterin, it is perhaps accumulated as one of the least soluble products of their

¹ Fischler, *Cent. f. Path.*, 1904 (15), 913.

² Ziegler's *Beitr.*, 1905 (7th suppl.), 343.

³ *Jour. Exp. Med.*, 1905 (7), 633.

⁴ Wells, *Jour. Med. Research*, 1906 (14), 491.

⁵ Concerning the chemistry of cholesterin see introductory chapter.

⁶ See Bostroem, *Cent. f. Path.*, 1897 (8), 1.

⁷ *Pharm. Centralhalle*, 1902 (43), 357.

disintegration. The origin of the normal cell cholesterol is unknown. According to Stadelmann,¹ that which enters with the food is not absorbed, hence the considerable amounts that are constantly being thrown out by the bile and the sebaceous glands must be replaced by cellular activity. Cholesterol is generally considered, but without convincing proof, to be a product of proteid decomposition; if this is true, then the cholesterol found in disintegrating tissues may be formed from the cell proteids during their decomposition.² Apparently cholesterol crystals may be slowly removed, the chief factor probably being the giant-cells that are often found surrounding them.³ In general they behave as inert foreign bodies.

AMYLOID⁴

Virchow, in 1853, made the first study of the nature of the substance characteristic of "lardaceous" degeneration, and considered it to be a sort of animal cellulose, because it often became blue if treated with iodine followed by sulphuric acid. To this resemblance in staining reaction we owe the unfortunate, misleading, but generally used, name amyloid.⁵ It was but a few years (1859) before Friedreich and Kekulé showed that the substance in question was of proteid nature; their methods were very crude, but the main fact was soon better substantiated by Kühne and Rudneff (1865). Krawkow,⁶ however, in 1897 gave us the first good idea of the composition of amyloid substance through his amplification of Oddi's⁷ observation that amyloid organs contain *chondroitin-sulphuric acid*, finding that amyloid is a compound of proteid with this acid, similar to nucleoproteid, which is a compound of nucleic acid and proteid.

¹ Dissert., Freiburg in der Schweiz, 1898.

² Of historical interest is Austin Flint's idea that cholesterol in the blood is an important factor in intoxications, especially in icterus (Amer. Jour. Med. Sci., 1862 (44), 29). All recent evidence is to the effect that cholesterol is not toxic.

³ See LeCount, Jour. Med. Research, 1902 (7), 166.

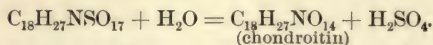
⁴ General literature to 1893, see Wichmann, Ziegler's Beitr., 1893 (13), 487; also Lubarsch, Ergeb. allg. Path., 1897 (4), 449; modern ideas are summed up in a discussion in the Verh. Deut. Path. Gesellsch., 1904 (7), 2-51.

⁵ In view of the fact that this substance is chemically related to chondrin, and that it also closely resembles this substance physically, it has seemed to the writer that the name "chondroid" would be much more appropriate than any of the many more or less misleading and inappropriate titles that are at present in use. The very multiplicity of these terms, however, prohibits any attempt to introduce still another. A particularly unfortunate source of confusion exists in the use of the name amyloid for a vegetable substance, formed by the action of acids upon cellulose.

⁶ Arch. exp. Path. u. Pharm., 1897 (40), 196.

⁷ *Ibid.*, 1894 (33), 377.

Chondroitin-sulphuric acid, which has been studied especially by Mörner and by Schmiedeberg,¹ has the formula $C_{18}H_{27}NSO_{17}$, according to the latter, and yields on cleavage *chondroitin* and sulphuric acid, as follows :



Chondroitin is a gummy substance which in turn may be split into acetic acid and a reducing substance, *chondrosin*. Chondroitin-sulphuric acid is the characteristic component of cartilage, but it is also found in mucin (Levene), and in the walls of the aorta and other elastic structures (Krawkow). It has also been found in a uterine fibroma and in bone tissue by Krawkow, but could not be found in the parenchymatous organs, normal and pathological, or in chitinous structures. Mörner has also found it in a chondroma.

Chemistry of Amyloid.—Krawkow separated amyloid from nucleoproteid, to which it is most closely related, by dissolving both substances from the minced amyloid organs with ammonia, precipitating with acid, and then taking up the amyloid with $Ba(OH)_2$ solution, in which the nucleoproteid does not dissolve. Amyloid thus isolated is a nearly white powder, which is easily soluble in alkalis, but slightly in acids, and is very resistant to pepsin digestion. The elementary composition was found by Krawkow to be approximately as follows :

C=49–50%; H=6.65–7%; N=13.8–14%; S=2.65–2.9%; P in traces only.

Quite similar analytic results have been obtained by Neuberg,² who corroborated Krawkow's finding of a body of apparently similar composition in the normal aorta. Neuberg has studied especially the proteid constituent of the amyloid compound, and found it characterized by a high proportion of diamino-nitrogen, as compared with most proteids, as shown in the following table giving the percentage of the total N contained in each of the three forms, amid-nitrogen (ammonia), monamino-acids, and diamino-acids :

TABLE I.

	Monamino- acid nitrogen.	Diamino- acid nitrogen.	Amid nitrogen.
Liver amyloid	43.2	51.2	4.9
Spleen amyloid	30.6	57.0	11.2
Aorta "amyloid"	54.9	36.0	8.8
Gelatin	62.5	35.8	1.6
Casein	76.0	11.1	13.4

¹ Mörner, Skand. Arch. Physiol., 1889 (1), 210; Zeit. physiol. Chem., 1895 (20), 357, and 1897 (23), 311; Schmiedeberg, Arch. exp. Path. u. Pharm., 1891 (28), 358.

² Verh. Deut. Path. Gesell., 1904 (7), 19.

The variations in the composition of the different amyloids, as shown in the above table, indicate that the proteid group may vary in different organs or in different cases, and also indicate that the "amyloid-like" substance of normal vessels is not the same as the pathological substance. Corresponding variations were found in the apportionment of the sulphur between that which is in the form of oxidized sulphur and the unoxidized sulphur. The proportion of the different amino-acids in the proteid constituent of amyloid is strikingly like that of thymus histon, and entirely dissimilar to the apparently closely related elastin, as shown by the following table :

TABLE II.

	Cleavage products (in percentages).		
	Amyloid.	Elastin.	Thymus histon.
Glycocoll	0.8	25.8	0.5
Leucin	22.2	45.0	11.8
Glutaminic acid	3.8	0.7	3.7
Tyrosin	4.0	0.3	5.2
α -Prolin	3.1	1.7	1.5
Arginin	13.9	0.3	14.5
Lysin	11.6	..	7.7

This carries out the resemblance of amyloid to nucleoproteids, and, likewise, Neuberg found amyloid very slowly digested by pepsin, and much better by trypsin, although less rapidly than simple proteid ; it is also destroyed by autolytic enzymes, for amyloid tissues readily undergo autolysis. Neuberg considers, from the above results, that amyloid is probably a transformation-product of the tissue proteid, similar to the transformation of simple proteids into protamins that occurs in the testicle of spawning salmon as they go up the streams, as shown by Miescher's classical studies.

Krawkow considers that amyloid differs from normal chondroitin-sulphuric acid compounds, such as cartilage, in that in the latter the acid radical is in a loose combination with the proteid, while in amyloid the combination is a very firm one, perhaps in the nature of an ester. The occurrence of the typical amyloid reaction in what appears otherwise to be normal cartilage, occasionally observed in senile tissues, may be due to the transformation of loosely bound into firmly bound chondroitin-sulphuric acid. In any event, amyloid is not essentially a pathological product,

but rather a slightly modified normal constituent of the body.

Staining Properties.—The classical reaction for amyloid is its staining a reddish brown when treated with iodine (best as Lugol's solution) in the fresh state. Such stained specimens, if afterward treated with dilute sulphuric acid, usually become blue or greenish, but may merely turn a deeper brown. Occasionally old compact amyloid may stain bluish or green with iodine alone. The iodine reaction disappears in specimens that have been kept for some time in preserving fluids, or in tissues that have become alkaline, and is generally less persistent than the metachromatic staining by methyl-violet or methyl-green, which color the amyloid red. Occasionally an otherwise typical amyloid will fail to react to iodine, but will stain well with methyl-violet. All these variations may occur in different specimens from the same body, and the blue iodine-sulphuric acid reaction is usually given well only by splenic amyloid. These variations probably depend upon the age and stage of development of the amyloid, or upon secondary alterations, and are perhaps related to Neuberg's observations on the difference in composition of amyloid of different origins.

Krawkow studied these reactions with pure, isolated amyloid, and found evidence that the iodine reaction depends upon the physical properties of the amyloid, while the methyl-violet stain is a chemical reaction, and hence the iodine reaction is much the more readily altered or lost. As Dickinson¹ says, amyloid stains with iodine simply as if it absorbed the iodine more than does the surrounding tissue. The methyl-violet reaction is due to the dye forming a compound with the chondroitin-sulphuric acid, for Krawkow found that these substances unite with one another to form a rose-red precipitate. Schmidt found that implanted pieces of amyloid lost their iodine reaction as they underwent autolysis, while the methyl-violet reaction was still very distinct.² It is evident, therefore, that iodine is not by itself a specific stain for amyloid, especially as glycogen gives a similar reaction,³ while true amyloid may not react.

¹ Allbutt's System, vol. 3, p. 225.

² Litten (Verh. Deut. Path. Gesell., 1904 (7), 47) states that thionin and kresyl-violet are the most specific stains for amyloid, which they color blue; whereas methyl-violet stains red not only amyloid but also mucin, mast cell granules, and the ground substance of cartilage. v. Gieson's stain usually colors amyloid pale yellow, and hyalin red.

³ See Wichmann, Ziegler's Beitr., 1893 (13), 487.

THE ORIGIN OF AMYLOID

This question has not been at all cleared up as yet by the advances made in our knowledge of the chemistry of amyloid substance. The fact that chondroitin-sulphuric acid is a characteristic constituent suggests that this body may be liberated in considerable amount during the destructive processes to which amyloidosis is usually secondary; this idea is further supported by the fact that amyloidosis occurs particularly after chronic suppuration in bone and lungs, both of which tissues, according to Krawkow, contain chondroitin-sulphuric acid. This idea was not substantiated, however, by the experiments made by Oddi and by Kettner,¹ who fed and injected into animals large quantities of the sodium salt of chondroitin-sulphuric acid without producing amyloid changes. Unpublished experiments of the writer with the same material, as well as with ground-up cartilage and with mucin, were equally unsuccessful. As it is possible to cause amyloidosis experimentally in animals, especially chickens and rabbits, by causing protracted suppuration or chronic intoxication with bacterial filtrates, these negative results speak strongly against the idea of a transportation of chondroitin-sulphuric acid, but do not determine it finally. Especially important in this respect is the extreme difficulty of producing amyloid experimentally, for in only a certain proportion of cases are the experiments positive (in but about one-third of Davidsohn's² 100 trials; and many other experimenters have been much less successful³). Davidsohn, failing always to get amyloid experimentally after the spleen had been removed, suggests that this organ (in which amyloid is usually earliest and most abundantly observed) produces an enzyme, which causes a precipitation of amyloid in the tissues from a soluble precursor brought in the blood from the site of cell destruction. Schmidt⁴ gives an excellent discussion of the various features of amyloidosis, and also considers it probable that some enzymatic action causes a precipitation or coagulation of some substance in the tissue-spaces or lymph-vessels. Amyloid is never deposited in the cells themselves, and it seems to be now generally considered that the amyloid material is infiltrated in the form of a soluble modification or precursor, and that it is not manufactured in the organ where it is found. It is an interesting fact that a

¹ Arch. exp. Path. u. Pharm., 1902 (47), 178.

² Verh. Deut. Path. Gesell., 1904 (7), 39.

³ See Tarchetti, Deut. Arch. klin. Med., 1903 (75), 526.

⁴ Verh. Deut. Path. Gesell., 1904 (7), 2.

practically identical substance is formed in all tissues and in all species of animals, even when the cause is quite different. Whether the precursors are brought to the organ in solution, or in leucocytes, is unknown—probably the former. The hypothesis that amyloid is formed from disintegrating red corpuscles is probably incorrect. It is also quite certain that it is not of bacterial origin, for amyloidosis has been produced by chronic intoxication with aseptic materials (rennin),¹ and it is also produced by the most varied species of bacteria and by their toxins, although the staphylococcus is usually most effective in experimental work.² Neither is suppuration absolutely essential, for injection of toxins alone (*e. g.*, in preparing diphtheria antitoxin³), without suppuration, may produce amyloidosis, as also frequently do syphilis without suppuration and, less often, many other non-suppurative conditions (*e. g.*, tumors).

Local amyloid accumulations⁴ are of some interest in considering the genesis of the usual generalized form. They occur particularly as small tumors in the larynx, bronchi, nasal septum, and eyelids; as all these tissues are normally rich in chondroitin-sulphuric acid, it seems probable that the amyloid arises from a local overproduction of chondroitin-sulphuric acid, which becomes bound with proteids *in situ*. This makes it seem more probable that, in spite of the lack of positive experimental evidence, general amyloidosis is due to liberation of excessive quantities of chondroitin-sulphuric acid in the sites of tissue destruction.

Another form of local amyloid is seen particularly in the regional lymph-glands of suppurating areas; *e. g.*, the lumbar glands in vertebral caries, the axillary glands in shoulder-joint suppuration. This local amyloidosis is undoubtedly due simply to the fact that these glands receive first, and in largest amounts, the cause, whatever it may be, of the amyloid production.⁵

Corpora amylacea will be found discussed under "Concretions" (Chap. xv).

¹ Schepilewsky, *Cent. f. Bakt.*, 1899 (25), 849.

² In a series of experiments directed to ascertain, if possible, which constituent of pus might be the cause of amyloid formation, I was unable to secure amyloid by protracted intoxication of rabbits by Witte's "peptone," which consists chiefly of proteoses (*Trans. Chicago Path. Soc.*, 1903 (5), 240).

³ Zenoni, *Riforma Med.*, 1901 (2), 698.

⁴ See Edens, *Virchow's Arch.*, 1905 (180), 346.

⁵ Quite unexplained is the cause of the rarely observed localization of amyloid in the wall of the urinary bladder. See Lucksch (*Verh. Deut. path. Gesell.*, 1904 (7), 34).

HYALINE DEGENERATION¹

Much confusion concerning this condition may be avoided if we appreciate that the term hyaline indicates a certain physical condition, which may be exhibited by many substances of widely different nature and origin. *There is no one chemical compound, "hyalin,"* which, accumulating in cells or tissues, produces a hyaline appearance. The limits of the application of the term "hyaline degeneration," even to histological findings, is not agreed upon, but in general it is used to apply to clear, homogeneous, pathological substances that possess a decided affinity for acid stains, such as eosin or fuchsin. Somewhat similar substances, usually of epithelial origin, which do not take either these or basic stains strongly, are usually called "colloid." We may properly consider that pathological hyalin can be divided into two chief classes according to its origin: (1) connective-tissue hyalin; (2) epithelial hyalin.

Connective-tissue hyalin is characterized, like amyloid, by being deposited in or among the fibrillar substance of connective tissues, and not within the cells themselves, but there are undoubtedly several different sorts of chemical substances responsible for various forms of connective-tissue hyalin. One form is closely associated with amyloid, being found in organs showing amyloid degeneration, or in other tissues in the same body. In experimentally produced amyloidosis in animals it has been shown that such a hyaline substance may appear before the amyloid, which eventually replaces it; hence, it has been suggested that hyalin is a precursor of amyloid. Such hyalin differs from true amyloid only in its failure to give the characteristic staining reaction of amyloid; in all other respects, *e. g.*, cause, location, termination, it is the same. As it has been shown (see preceding section) that the staining properties of amyloid are very inconstant, it is probable that the above-described variety of hyalin is merely an *incompletely developed*, or occasionally a *retrogressively altered* amyloid. However, it is probably not necessary, as some authors have thought, that amyloid should always pass through this hyaline stage in its formation.

Quite different, without doubt, is the form of hyalin observed in *scar tissue*. This variety develops almost constantly in any scar-tissue after the blood-supply has been reduced to a minimum through contraction, and is seen characteristically in the corpora fibrosa of the ovary, fibroid glomerules in chronic

¹ General literature, see Lubarsch, *Ergeb. allg. Path.*, 1897 (4), 449.

nephritis, thickened pleural, pericardial, and episplenitis scars, etc. Such hyaline substance occurs independent of the usual causes of amyloid, affects only abnormal fibrous tissue, never changes into amyloid, and is prone to undergo calcification—it surely has no close chemical relation to the form of hyalin that does become amyloid. Presumably, it is similar in nature to the collagen of normal fibrous tissue intercellular substance, which has undergone physical rather than chemical changes into a homogeneous hyaline substance. For its physiological prototype it has the thick “collagenous” fibers of the subcutaneous connective tissue.

Probably of quite different origin is the hyalin that develops from elastic tissue, as seen best in the thick-walled, partly obliterated arteries of the senile spleen; and less characteristically in the early stages of arteriosclerosis, since here the preceding form of connective-tissue hyalin may also occur. Although arterial elastic tissue is related chemically to amyloid, these hyaline vessels do not develop the usual amyloid reaction, but remain more or less of the specific, elastic tissue stains. Presumably this form of hyalin is an increased and physically altered elastin.¹

Epithelial hyalin occurs within the cells, and includes substances of presumably widely diverse chemical nature, from the keratin of squamous epithelium to the small intracellular hyaline granules of carcinoma and other degenerating cells (Russell’s fuchsin bodies²). Extracellular substances of hyaline character, but of unknown composition, may also be produced by epithelium; *e. g.*, hyaline casts in the renal tubules.

Many other pathological materials of widely differing nature may, under certain conditions, assume a hyaline appearance; *e. g.*, fibrinous exudates and thrombi, degenerated muscle-fibers (Zenker’s or “waxy” degeneration), tumor-cells (cylindroma), etc. In all of these the chemical nature of the parent substance or substances is probably much less altered than its physical appearance, but whether the change is related to the process of proteid coagulation or not is unknown.

COLLOID DEGENERATION

This term, also, has a very indefinite meaning, and is applied to many different conditions by various authors. Thus, v. Recklinghausen includes under this name amyloid, epithelial hyaline, and mucoid degeneration. Marchand includes hyaline connective-tissue degeneration, and, also, as do most other

¹ See Schmidt, Verh. Deut. path. Gesell., 1904 (7), 2.

² Literature, see Hektoen, Progressive Med., 1899 (ii), 241.

writers, the mucoid degeneration of carcinoma. Ziegler rightly protests against the inclusion of mucin under this heading, but includes the corpora amylacea. On account of the discovery by Baumann of the specific chemical nature of thyroid colloid it becomes particularly unfortunate that the term "colloid" has such a wide and uncertain application. It would seem that the safest view to take is that *the word colloid is merely morphologically and macroscopically descriptive* of certain products of cell activity or disintegration, which have nothing in common except the fact that they form a thick, glue-like or gelatinous, often yellowish or brownish substance. *There is no one definite substance colloid*, according to the usual usage of the word in pathological literature, but many different proteid substances may assume the appearance to which the name "colloid" is given. Looking at the matter in this way, we must recognize as the usual "colloid" substances, the following chemical bodies:

Thyroid colloid, the physiological prototype of the group. This consists of a compound of globulin with an iodine-containing substance, thyroiodin, the compound proteid being called by Oswald iodothyreoglobulin. It occurs pathologically only in cystic and similar changes in the thyroid or accessory thyroids. Being a specific product of the thyroid (and perhaps of the hypophysis) with definite physiological properties, it manifestly has only a morphological relation to the other forms of colloid found in degenerating tumors, etc. (The nature of thyroid colloid is discussed more fully under "Diseases of the Thyroid," Chap. xx.)

Mucin, when secreted in closed cavities, as in tumors, where it becomes thickened by partial absorption of the water, may take on a "colloid" appearance while retaining its chemical and tinctorial characteristics. This is particularly observed in the "colloid" carcinomas which arise especially from the mucous membrane of the alimentary tract. This substance is, of course, quite specific both in its chemical nature and its origin from specialized epithelial cells, and the process should properly be considered as a "mucoid degeneration."

Pseudomucin, which differs from mucin in not being precipitated by acetic acid, is a common component of ovarian cysts, and when somewhat concentrated by absorption of water, forms a "typical colloid." Because it is alkaline, this form of colloid tends to stain rather with the acid dyes (eosin, fuchsin, etc.), while true mucin stains with basic dyes. Several varieties of pseudomucin have been described by Pfannenstiel, and their

properties will be considered more fully in the section on "Ovarian Tumors" (Chap. xvii). The clear, glassy, yellowish substance contained in small cavities of ovarian tumors, which is usually called "colloid," consists of nearly pure pseudomucin. All these substances yield a reducing substance on boiling with acids, which is a nitrogen-containing body, *glucosamin*.¹

Simple proteids (*e. g.*, serum-globulin, serum-albumin, nucleo-albumin, etc.) may, when in solution in closed cavities, become concentrated through absorption of water until they produce the physical appearance of "colloid." Probably the colloid contents of dilated renal tubules, cavities in various mesoblastic tumors, etc., are produced in this way.

MUCOID DEGENERATION

Mucin, in its typical form, is a compound proteid, consisting of a proteid radicle and a nitrogen-containing carbohydrate, *glucosamin*. Hence, when boiled with acids, mucin yields a substance reducing Fehling's solution. Mucin is acid in reaction, probably because of the presence of chondroitin-sulphuric acid (at least in some varieties of mucin), and, therefore, is characterized microchemically by staining with basic dyes. It is readily dissolved in very weak alkaline solutions, is precipitated by acetic acid, and its physical properties when in solution are quite characteristic. The term mucin, however, probably covers a number of related but distinct bodies. Some, such as the *pseudomucins*, are readily distinguished by not being precipitated by acetic acid, and by being alkaline in reaction; others yield reducing substances without previous decomposition with acids (paramucin); while even among the "true" mucins certain differences in solubility exist.²

In the mammalian body we find mucin occurring in two chief localities: (1) as a product of secretion of epithelial cells; (2) in the interstices of connective tissue, especially of tendons. (The resemblance of synovial fluid to mucin is more physical than chemical.) There is also evidence that mucin or a related body constitutes the cement substance between all the body-cells. Corresponding to these two chief sources of mucin we find mucoid degeneration occurring as distinct processes in mucous membranes (or tissues derived therefrom) and in connective tissue.

¹ Zängerle, Münch. med. Woch., 1900 (47), 414.

² For special consideration see Cutter and Gies, Amer. Jour. Physiol., 1901 (6), 155.

Epithelial Mucin.—As epithelial mucin represents a distinct product of specialized cells, it is questionable if the ordinary application of the term degeneration, in the sense of the conversion of cell-protoplasm into mucin, is correct. Certainly the mucin formation of catarrhal inflammation is merely an excess of a normal secretion, and the degenerative changes that may be present in the epithelial cells are produced by the cause of the inflammation, and are not dependent upon mucin formation. Even in the extreme example of mucoid degeneration seen in carcinomas derived from mucous membranes (the so-called "colloid cancers"), the epithelial degeneration is not necessarily to be interpreted as a conversion of cell-cytoplasm into mucin, but is largely due to the pressure of secreted mucin upon the cells within the confined spaces of the tumor. The mucin in these forms of mucoid degeneration is chemically the same as the normal mucin coming from the same source, but mixed with larger or smaller quantities of other proteids derived from cell degeneration or from vascular exudates. (The stringy, mucin-like substance seen in some purulent exudates is probably composed largely of nucleoproteids and nucleo-albumins derived from the degenerating leucocytes, and is not true mucin.)

Connective-tissue Mucin.—Excessive formation of connective-tissue mucin is observed most characteristically in myxedema (*q. v.*), but may also occur in connective tissues that are poorly nourished or otherwise slightly injured; it is seen particularly in the connective tissues surrounding the epithelial elements in adenomas and carcinomas. Connective-tissue tumors (myxosarcoma, myxofibroma, or myxoma) may also show a great quantity of mucinous intercellular substance, but many of the so-called myxomas are in reality merely edematous fibromas or polypoid tumors, in which the resemblance to true myxoma is largely structural rather than chemical. This form of mucoid degeneration seems to be merely a reversion to the fetal type of connective tissue, which is characterized, as in the umbilical cord, by an excessive accumulation of a mucin-containing fluid intercellular substance, and a paucity of collagenous fibrillar structure. Apparently, when connective tissue reverts to an embryonal type, either from intrinsic causes (tumor formation), or when the nourishment is insufficient, or possibly when the normal stimulus to cell growth is absent (myxedema), the mucoid characteristics of fetal tissue reappear.

The presence of mucin in the tissues seems to cause no reaction, and its absorption causes no harm. Rabbits that I injected with large quantities of pure tendon mucin almost daily

for two to four months, showed absolutely no deleterious effects, either locally or constitutionally.¹ Some of the French authors² claim that mucin possesses a slight bactericidal power. On the other hand, Rettger³ and others have found an apparently typical mucin produced by certain varieties of bacteria.

GLYCOGEN IN PATHOLOGICAL PROCESSES

It seems probable that all, or nearly all, cells contain larger or smaller quantities of glycogen, but it may be insufficient in amount to be detected either microscopically or chemically. Glycogen seems to be formed within the cells from the sugar of the blood, through a process of dehydration and polymerization, and to be reconverted whenever necessary into sugar, by a reverse process of hydrolysis. It is quite possible that both of these processes represent merely the reversible action of an intracellular enzyme, but this has not been established. We do know, however, that soon after death the intracellular glycogen is rapidly converted into dextrose.⁴

Properties of Glycogen.—Glycogen is frequently called an "animal starch," having the same general composition as the starches ($C_6H_{10}O_5$)_x, and apparently, like the starches, it represents a relatively insoluble resting stage of sugar in the course of metabolism. It is readily soluble in water, forming an opalescent, colloidal solution, and, therefore, has no effect on osmotic pressure, and it is not diffusible.⁵ Because of its solubility and the rapidity with which postmortem change to dextrose occurs, specimens that are to be examined microscopically for glycogen must be hardened while very fresh in strong alcohol, in which glycogen is insoluble.⁶ One of the most characteristic reactions is the port-wine color given by glycogen when treated with iodine; this reaction may be applied microscopically, solution of the glycogen being avoided by having the iodine dissolved in a solution of gum arabic or in glycerin. Salivary ptyalin rapidly converts glycogen into glucose, and this reaction may also be used microscopically to prove that suspected granules are glycogen.

¹ Levin (Med. Record, 1900 (57), 184) claims that mucin injected into thyroidectomized rabbits is very poisonous for them, while not harming normal rabbits.

² Arloing, *Compt. Rend. Soc. Biol.*, 1902 (54), 306, and 1901 (53), 1117.

³ *Jour. Med. Research*, 1903 (10), 101.

⁴ Literature concerning physiology of glycogen by Pflüger, *Pflüger's Arch.*, 1903 (96), 398; and Cremer, *Ergeb. der Physiol.*, 1902 (1, Abt. 1), 803.

⁵ See Gatin-Gruzewska, *Pflüger's Arch.*, 1904 (103), 282.

⁶ According to Helman (*Cent. f. inn. Med.*, 1902 (23), 1017), glycogen may be found in specimens preserved in alcohol as long as fifteen years.

PHYSIOLOGICAL OCCURRENCE

According to Gierke,¹ the normal glycogen of cells resembles fat in that part of it disappears during starvation, while the rest cannot be removed in this way and probably is something more than a reserve food-stuff. In distribution glycogen somewhat resembles fat, being abundant in the liver² and muscles, but Gierke considers that the microscopic evidence of the quantity of glycogen present in the cell agrees better with the results of actual chemical analysis than is the case with fat. Neither iodine nor Best's carmin stain are absolutely specific for glycogen, but Gierke believes that we may safely consider a substance as glycogen when it is homogeneous, rather easily soluble in water and more so in saliva, gives the usual iodine reaction, and stains bright red with Best's carmin solution.³ With these controls, the microscopic findings were found to agree closely with the results of direct chemical analysis, and glycogen was found microscopically visible in muscle, liver, lung, heart, uterus, and skin (but not in the brain, where it may be demonstrated chemically in minute quantities).

Glycogen is especially abundant in fetal tissues, but it is not present in all fetal cells, nor is it always most abundant in the most rapidly growing tissues. Although both fat and glycogen are quite abundant in fetal muscle and liver tissues, the liver of early embryos does not contain either.⁴ In extra-uterine life glycogen is relatively less abundant; invertebrates and the lower vertebrates have more than the higher forms. In mammalian adults the liver and muscle contain the most glycogen, cartilage standing next, and it is also present in squamous epithelium (particularly the middle layers), but not in slightly stratified (cornea), transitional, or cylindrical epithelium. The normal lung is microscopically glycogen-free (except for its cartilage and muscle), as also are the nervous system, pancreas, salivary glands, thyroid, hypophysis, bone-marrow, and adrenals; normal human kidneys do not seem to show glycogen, but it may be present in the kidneys of mice, rabbits, and cats.

¹ A complete summary of all the literature to the end of 1904 is given in Gierke's article in Ziegler's Beitr., 1905 (37), 502; hence references included by Gierke will not generally be given.

² In the livers of two executed criminals Garnier (Compt. Rend. Soc. Biol., 1906 (60), 125) found respectively 4 per cent. and 2.79 per cent. of glycogen.

³ A special staining method is recommended by Driessen, Cent. f. Path., 1905 (16), 129.

⁴ Adamoff (Zeit. f. Biol., 1905 (46), 288) contests the idea that the amount of glycogen is in direct relation to growth energy.

There is very little in heart muscle or testicle, and none in the ovaries and corpus luteum or in the mammary glands, although it may be present in their fat-cells. Glycogen is most abundant in the uterus at the time of child-birth, and is abundant in the placenta. After pancreas extirpation, Fichera¹ observed a disappearance of all visible glycogen, except a little in the cartilage and stratified epithelium; hence he considers the glycogen-content as a function of cell nourishment. Fat and glycogen often occur together (which is contrary to Rosenfeld's statement), although one may be present without the other (Gierke).

There has been some diversity of opinion as to whether glycogen occurs as granules in the living cell, or whether the granules are formed from a homogeneous substance by hardening fluids. In view of the clear-cut, definite spaces it may leave in cells when dissolved out, glycogen probably occurs as granules, especially when present in abnormally large quantities. It has been suggested that the intraepithelial hyaline bodies (Russell's fuchsin bodies) are glycogenic, which idea is probably not correct. Habershon has also suggested that eosinophile granules are either glycogen or related to it. The presence of glycogen in the cells seems to cause no injury to the cytoplasm, and if it again disappears, the cells become quite normal.²

GLYCOGEN IN PATHOLOGICAL PROCESSES

According to the results obtained by Fichera and Gierke, it seems probable that glycogen accumulation is produced under the same conditions as are fatty changes; *i. e.*, when oxidation is locally or generally impaired. Fat and glycogen are, therefore, often found together in the margins of infarcts and of tubercles, and in heart muscle with fatty changes due to severe anemia. The glycogen, being more labile, seems to disappear early when the cells become necrotic, and hence glycogen is not present in older necrotic areas where the fat still persists. (This probably accounts for the frequently repeated statement that glycogen and fat do not occur together.) Whether the glycogen can be transformed into fat, perhaps forming an intermediary stage in a transformation of proteid into fat, has not been determined, but there seems to be little doubt that it is infiltrated

¹ Ziegler's Beitr., 1904 (36), 273, literature.

² Yet Teissier (Compt. Rend. Soc. Biol., 1900 (52), 790) believes the amount normally present in the liver is strongly bactericidal, and in a later publication (*ibid.*, 1902 (54), 1098) considers that it is toxic to liver-cells. Wendelstadt (Cent. f. Bact., Abt. 1, 1903 (34), 831) found that under certain conditions glycogen impedes hemolysis by normal serum.

from outside the cell, and not formed directly from degenerated proteid. It seems to be deposited only in cells that are still living, although it can become split up in dead cells. All cells, but especially muscle-cells and leucocytes, seem able to lay up glycogen in visible amounts under certain conditions. In inflamed areas glycogen is found both in tissue-cells and leucocytes, but not in cells showing nuclear degeneration (Best, Gierke). In pneumonia the leucocytes of the exudate, and to a less extent the alveolar epithelium, contain glycogen as well as fat.

Glycogen in Tumors.—Glycogen has been observed frequently in tumors. Brault believed the quantity an index of rate of growth, on the principle that glycogen appears most abundantly in embryonal tissues, and therefore in tumors the amount of glycogen should agree with the degree to which the cells have gone back to the embryonic type. Lubarsch considered that only tissues normally containing glycogen give rise to glycogen-containing tumors. Gierke could corroborate neither of these ideas, and considers that glycogen arises in tumors under exactly the same conditions in which it arises in other tissues; *i. e.*, when cell nutrition and oxidation are impaired. Apparently, however, *both the embryonic origin and local retrogressive changes determine the deposition of glycogen in tumors.* Glycogen is particularly abundant in squamous epithelium of epitheliomas that have gone on to hornification; in testicular tumors, hypernephromas, endotheliomas, chondromas, and myomas, and it also occurs in the connective tissues surrounding tumors. Of 1544 tumors of all sorts examined by Lubarsch,¹ 447 (or 29 per cent.) contained glycogen microscopically; fibromas, osteomas, gliomas, hemangiomas were always free from glycogen; and lipomas and lymphangiomas nearly always. Adenomas are almost equally free from glycogen (two positive in 260 specimens), while it was constant in teratomas, rhabdomyomas, hypernephromas, and chorioepitheliomas. Fifty and seventenths per cent. of the sarcomas and 43.6 per cent. of the carcinomas show glycogen, most abundant in squamous-cell epitheliomas; columnar-celled carcinomas contain glycogen much less often, and it is always absent in "colloid cancers."

Animal parasites, in common with other invertebrates, usually show abundant quantities of glycogen.² It has been

¹ Virchow's Arch., 1906 (183), 188.

² Elaborate treatise on occurrence of glycogen in lower animals by Barfurth, Arch. mikros. Anat., 1885 (25), 269; also Busch, Arch. internat. physiol., 1905 (3), 49; Brault and Loeper, Jour. Phys. et Path. Gén., 1904 (6), 295 and 720.

found in protozoa, as well as in all varieties of intestinal worms. According to Barfurth, nematodes in glycogen-free animals may contain glycogen. The glycogen is found chiefly in the connective tissues of the intestinal parasites, but in some of the nematodes it occurs chiefly in the sexual organs and muscle-cells. The walls of hydatid cysts contain much glycogen, which is, perhaps, related to the usual presence of sugar in their contents. If Habershon's contention is correct, that eosinophile granules are related to glycogen, we may have here an explanation of the occurrence of eosinophilia in infection with animal parasites. (See also "Animal Parasites," Chap. v.)

Glycogen in Leucocytes.—The occurrence of glycogen in the blood has aroused much interest, particularly in relation to its diagnostic value. Many leucocytes contain granules that stain with iodine, and although it is possible that these are not all granules of glycogen, yet, for the most part, they probably represent this substance in excessive quantities. The granules are observed chiefly in the polymorphonuclear neutrophils, but also in large and small mononuclear cells; only in diabetes do the eosinophiles contain glycogen, according to most authors, but Habershon believes that eosinophile granules are related to or identical with glycogen. Occasional granules are also found free (or perhaps contained in blood-platelets) in all blood, whether normal or pathological,¹ whereas, according to Locke, the leucocytes contain the granules only in pathological conditions. It does not seem to be settled whether the glycogen is taken on by the leucocytes at the place of pathological lesion, or in the bone-marrow under the influence of circulating poisons, or both. Habershon states that from 1 to 16 per cent. of all leucocytes normally contain glycogen granules, and Wolff believes that the glycogen seen in leucocytes represents normal glycogen made insoluble through injury.

Locke gives the occurrence of this abnormal iodine staining of the leucocytes (termed *iodophilia*) as follows: "Septic conditions of all kinds, including septicemia, abscesses, and local sepsis, except in the earliest stages, appendicitis accompanied by abscess formation or peritonitis, general peritonitis, empyema, pneumonia, pyonephrosis, salpingitis with severe inflammation or abscess formation, tonsillitis, gonorrheal arthritis, and hernia or acute intestinal obstruction where the bowel has

¹ Literature—Locke and Cabot, Jour. Med. Research, 1902 (7), 25; Locke, Boston Med. and Surg. Jour., 1902 (147), 289; Reich, Beitr. klin. Chir., 1904 (42), 277; Küttner, Arch. klin. Chir., 1904 (73), 438; Gulland, Brit. Med. Jour., 1904 (i), 880; Habershon, Jour. Path. and Bact., 1906 (11), 95; Wolff, Zeit. klin. Med., 1904 (51), 407.

become gangrenous, have invariably given a positive iodophilia, and by its absence all these cases can be ruled out in diagnosis. In other words, no septic condition of any severity can be present without a positive reaction. Furthermore, the disappearance of the glycogen granules in the leucocytes in from twenty-four to forty-eight hours following crisis with frank resolution in pneumonia, and the thorough drainage of pus in septic cases, is of considerable importance."

In **exudates** glycogen is found in the leucocytes as long as they retain their vitality, but disappears soon after retrogressive changes begin; hence it is not usually present in sterile pus. Loeper¹ made quantitative estimates of the glycogen in exudates, finding from 0.59–0.62 gram per liter in cellular pneumococcus pleural effusion, 0.25 gm. in cellular tuberculous effusion, but only traces in serous tuberculous effusion and in an old tuberculous pyothorax. A pneumonic lung contained 0.85 gm. of glycogen per kilo, and traces were found in pneumonic sputum and in the contents of tuberculous cavities. When glycogen solution (1 per cent.) was injected into the peritoneal cavity, the endothelial cells and invading leucocytes became loaded with glycogen granules.

Glycogenic Infiltration in Diabetes.—It is in diabetes, however, that the most marked accumulations of glycogen are found, the granules frequently fusing in the cells into droplets larger than the nucleus; when dissolved out in ordinary microscopic preparations, the clear round space left is exactly like the space left by a fat-droplet, except that the margins show a tendency to take the basic stain for some unknown reason. In even the most extreme cases, however, the nucleus is well preserved. Glycogen is found particularly in the epithelium of Henle's tubules, in heart muscle, and in the leucocytes, whereas it is greatly diminished in the normal storehouses of glycogen, the liver and muscles. Fütterer describes masses of glycogen in the cerebral capillaries, resembling an embolic process. Sandmeyer analyzed the organs for glycogen in a case of diabetes, finding the following amounts in percentage of organ weight: liver, 0.613; kidneys, 0.1158; lungs, 0.0442; spleen, 0.07. Experimental diabetes (pancreas extirpation) produces a marked glycogenic infiltration.

¹ Arch. Méd. Exp., 1902 (14), 576.

CHAPTER XV

CALCIFICATION, CONCRETIONS, AND INCRUSTATIONS

CALCIFICATION ¹

Pathological calcification occurs in two forms: one is a precipitation of calcium in secretions and excretions of the body; the other is the deposition of calcium salts in the tissues themselves. The former, which includes not only concretions in general, but probably also the deposition of calcium salts in the cells and tubules of the kidney,² both in disease and in experimental calcification after certain poisonings, is readily enough explained in most instances by recognizable alterations in the composition of the secretions, which lead to simple chemical precipitations. With this form we shall deal in the subsequent consideration of concretions, but, in referring to calcification, shall indicate only depositions within the tissues.

Relation of Calcification to Ossification.—In normal ossification we have to deal with the accumulation of lime salts within the stroma or cells of a tissue that has usually undergone certain preparatory changes in the way of formation of a more or less homogeneous ground substance, but has not suffered a total loss of vitality, although vitality is possibly decreased. Pathological calcification is similar, in so far as we have to deal with deposition of much the same salts in tissues that have suffered either total or partial loss of vitality, and which very frequently indeed are hyaline. What appear to be essential differences are these: (1) In calcification the lime salts always remain in clumps and masses, often fusing to greater or less degree, but never with the diffuse even permeation of tissue seen in ossification. (2) All the cells within a calcified area, if not dead at the beginning of the process, eventually disappear for the most part, and we have sooner or later a perfectly inert mass, practically a foreign body, instead of a specialized tissue as in ossification. (3) Ossification is accomplished only in

¹ Literature and résumé: Pfaundler, *Jahrb. f. Kinderheilk.*, 1904 (60), 123; Wells, *Jour. Med. Research*, 1906((14), 491.

² See v. Kóssa, *Ziegler's Beitr.*, 1901 (29), 163.

varieties of connective tissue, but calcification may involve any sort of a cell, provided it is degenerated sufficiently.

Composition of the Deposits in Calcification.—The composition of the inorganic salts in calcified areas in the body seems to be practically the same, if not identical, whether the salts are laid down under normal conditions (ossification) or under pathological conditions. This may be shown by a table giving the proportion of inorganic salts found by analysis of normal bone, and the proportion found in calcified materials :¹

	Mg ₃ (PO ₄) ₂ .	CaCO ₃ .	Ca ₃ (PO ₄) ₂ .
PATHOLOGICAL CALCIFICATION.			
Bovine tuberculosis	0.84	12.8	85.9
“ “	0.9	13.1	85.4
“ “	1.2	11.7	86.4
“ “ (softened gland)	1.5	7.6	90.6
Human tuberculosis	1.2	10.1	87.8
Calcified nodule in thyroid	0.85	13.4	85.4
Thrombus, human	1.1	11.9	86.5
NORMAL OSSIFICATION.			
Human bone (Zalesky)	1.04	± 12.8	83.8
“ “ (Carnot)	1.57	10.1	87.4
“ “ (Carnot)	1.75	9.2	87.8
Ox bone (Zalesky)	1.02	.	86.1
“ “ (Carnot)	1.53	11.9	85.7

Iron may be present in pathological calcification as it is in ossification. According to Gierke,² in the fetus the entire skeleton contains iron as far as it has calcified, most at the points of active ossification. Iron was also found in the borders of a splenic infarct, in a thyroid with calcification of its secretion, in a kidney with calcification produced by sublimate poisoning, in calcified ganglion-cells of the brain, and in some psammomas and a psammosarcoma. On the other hand, Gierke could find no iron in calcified atheromatous arteries, lymph-glands, lung nodules, and common petrefaction strumas, or in tumors with bone formation as well as those with calcified degenerated particles. The significance of this iron and the nature of its union are both unknown. Pick³ considers that in certain forms of calcification of the vessels of the brain, the iron exists as a calcium-iron-albuminate, since calcified granules in the vessels that he studied gave the Berlin-blue reaction for iron, while

¹ Wells, *Loc. cit.*

² Virchow's Arch., 1902 (167), 318.

³ Neurol. Centralb., 1903 (22), 754.

after decalcification no coloration could be obtained. S. Ehrlich¹ states that elastic fibers in the vicinity of hemorrhages take up an iron-containing derivative of the blood-pigment, and this acts as a mordant for subsequent calcium deposition.

Structure of Calcified Areas.—As before mentioned, in calcification there is not the same uniform infiltration of the ground substance with lime salts that occurs in bone, yet the calcified area is possessed of a ground substance of organic material which does not dissolve in acids that remove the salts. There is no definite ratio between the lime salts and this albuminoid matrix, however. At first the salts occur in granules, which may become fused to a greater or less degree. It has been thought by some that the deposition occurs in the form of "*calcospherites*."

These are small calcareous bodies, usually of concentric structure, which were first described by Harting. They appear to occur widely distributed in normal tissues, both animal and plant, and seem to be the result of the formation of insoluble calcium salts in the presence of some organic substances, just as urinary and other concretions are formed about an organic nucleus. If calcium chloride and soluble carbonates are allowed to combine very slowly to form calcium carbonate in a solution of egg-albumen, these or indistinguishable bodies are formed, which on being dissolved are found to possess an organic stroma that exhibits a marked affinity for any pigmentary substance that may be present. Apparently, when the proper concentration exists, the salts in crystallizing hold between the crystals the albuminous substances by which they are surrounded. Dastre and Morat believe that the substratum is lecithin, which others have found occupying a similar place in prostatic concretions. Calcospherites have been found in tumors, in cystic cavities, and in bodies with beginning decomposition. It may be mentioned in passing that Littlejohn² observed the abundant formation of calcium phosphate crystals in bodies that had been immersed for some time in sea water. Oliver has found calcospherites in the tissues of a cancer of the breast. Pettit³ found calcospherites in a sarcoma of the maxilla, presenting insensible transitions into the substance of the osseous tissue, and he suggests the possibility that the calcospherite formation may be related to the formation of bone. It seems, however, that they are probably more related to the formation of the shells of invertebrates, which are largely composed of carbonates in crystalline structure with an organic ground substance between them, and very little phosphate indeed.

OCCURRENCE OF PATHOLOGICAL CALCIFICATION

As far as we know, calcification never occurs in normal tissue, except in the formation of bone. Often the infiltrated tissue is completely dead, as in infarcts, organic foreign bodies,

¹ Cent. f. Pathol., 1906 (17), 177.

² Edinburgh Med. Jour., 1903 (13), 127.

³ Arch. d. Anat. Micros., 1897 (1), 107.

caseous areas, and particularly in old inspissated collections of pus. It may be said that any area of dead tissue that is not infected, and that is so large or so situated that it cannot be absorbed, will probably become infiltrated with lime salts. Most frequently calcified, next to totally necrotic tissues, are masses of scar-tissue that have become hyaline subsequent to the shutting off of circulation in the scar by contraction of the tissue about the vessels. Elastic tissue also seems prone to an early calcification, and it is not uncommon to see the elastic laminae of small arteries calcified in an apparently selective manner. A peculiar form of calcification is that frequently found in ganglion-cells of the brain which have become degenerated or necrotic, particularly in the vicinity of old hemorrhages; the cells become infiltrated with lime salts until a complete cast of the cell, with dendrites and axis-cylinder well impregnated, is formed. The calcification of renal epithelium obtained experimentally by ligation of the renal vessels or by the administration of certain poisons, is considered by some to be more closely related to the formation of ordinary urinary concretions than to tissue calcification; in any event, because of the function of the renal tissues to excrete calcium, and the continuous bathing of the cells with calcium-containing urine, the conditions are quite different from what they are in ossification and other forms of pathological calcification. Calcification of epithelial cells does occur, however, and seems to be preceded by hyaline changes, in which hyaline substance the calcium is later deposited, as in epithelial pearls, for example.

LeNoir¹ attempts to lay down a law of calcification, as follows: "We know that certain pigments are fixed first in the tissues possessing the most feeble vitality. Charrin and Carnot have shown that mineral poisons (lead) accumulate by choice in tissue previously altered. The organism, therefore, seems to have a tendency to rid itself of valueless or toxic compounds in tissues where nutrition is least active. Lime salts do not form an exception to the general rule; if they are in excess in the blood, they accumulate in the cells that are necrobiotic, or in cells in which the vitality is feeble, and there are deposited in an insoluble condition." This law is expressed in rather too metaphysical a manner, but it probably contains a kernel of fact.

Metastatic Calcification.—What is perhaps the only exception to the rule that some form of tissue degeneration is required before calcification occurs is the "*metastatic calcification*"

¹ Bouchard's Path. Générale, vol. 3, pt. 2, p. 650.

of Virchow.¹ In conditions with much destruction of bone, as osteomalacia, caries, osteosarcoma, etc., deposits of lime salts have been found distributed diffusely in various organs, particularly in the lungs and stomach. As there is no evidence that these organs had been the site of any diffuse tissue necrobiosis before the calcification occurred, it seems probable that the deposits have been made in practically or quite normal organs, because of oversaturation of the tissue fluids by calcium salts. The fact that the lung and stomach, and also to a less degree the kidney, are picked out, suggests that the calcification is related to the fact that in these same organs we have the excretion of acids into their cavities, which leaves the fluids in the substance of the organs correspondingly alkaline, and an increase in the alkalinity of the fluids makes the calcium salts decidedly less soluble. Presumably, under normal conditions, the amount of calcium in the blood is too slight to be thrown down in this way, but when oversaturated because of the calcium absorption in the skeleton, precipitation occurs in the parts of the body where the alkalinity of the blood or tissue fluids is greatest. A number of cases similar to the metastatic form as to location and nature of the deposits have been observed unaccompanied by any bone absorption, which complicates the matter decidedly, and some writers² combat many of the prevailing ideas of metastatic calcification. Some have attempted to include the calcification of the vessels and other tissues in old age in the metastatic calcifications, ascribing the origin of the salts to the senile absorption of bone, but it is probably dependent rather upon the extensive hyaline degeneration of the connective tissues that occurs in the senile scleroses.

CHEMISTRY OF THE PROCESS OF CALCIFICATION

In analyzing the etiological factors in the production of pathological calcification for the purpose of determining the chemical changes that occur in the process, we have the following facts upon which to base the consideration :

(1) The calcium salts must come from the blood, where they are held in solution or in suspension by the proteids, either as the carbonate and phosphate themselves, or as calcium-ion-proteid compounds, or perhaps both. This suspension or solution is an unstable condition, possible only because of the extremely small proportion of calcium in the blood (about 1 : 10,000), and, there-

¹ Virchow's Arch., 1855 (8), 103; review by Kockel, Deut. Arch. klin. Med., 1899 (64), 332.

² Beer, Jour. Path. and Bact., 1903 (9), 225.

fore, capable of being overthrown by increased alkalinity of the blood, changes in the proteids, or changes in the quantity or composition of the calcium salts.

(2) Retrogressive changes in the tissues are a *sine qua non*. Hyaline degeneration, the chemical nature of which is not understood, is a very favorable condition, as also is necrosis when absorption is deficient.

(3) In the areas that are to become calcified the circulation is very feeble, the blood plasma seeping through the tissue as through any dead foreign substance of similar structure, without the presence of red corpuscles to permit of oxidative changes.

We may, therefore, imagine that the deposition of calcium salts in such areas of tissue degeneration depends upon any one of the following conditions :

(1) Increased alkalinity in the degenerating tissues, causing precipitation of the inorganic salts in the fluids seeping slowly through them.

(2) Utilization of the proteid of the fluids by the starved tissues so completely, because of its slow passage through them, that the calcium cannot be held longer in solution.

(3) The formation within the degenerated area of a substance or substances having a special affinity for calcium.

(4) Production of a physical condition favoring the absorption of salts, the least soluble salts accumulating in excess.

The first two ideas have little indeed to support them, and are mentioned chiefly because they have been advanced in the past by certain writers. The possibility of the formation of calcium-binding substances within the degenerated area has always seemed the most attractive, and has received the most attention by investigators. Of the special substances that might be present in such areas that would have a high affinity for calcium, *phosphoric acid* usually receives first consideration, since it is as phosphate that most of the calcium is bound, and also since the possible sources of phosphoric acid in decomposed nucleoproteids and lecithin are so obvious. Less considered in the past, *fatty acids* offer another possibility, especially in view of the fatty degeneration that so frequently precedes calcification. Proteids might also be formed that would combine calcium, especially deuterio-albumose, which Croftan¹ states has a high degree of affinity for calcium, and which would be present in areas undergoing autolysis.

¹ Jour. of Tuberculosis, 1903 (5), 22.

Formation of Calcium Soaps.—In favor of the possibility that the calcium is first bound as soaps are the following facts: Calcification occurs chiefly in places where fatty degeneration has occurred, such as tubercles, atheromatous vessels, etc. In fat necrosis fatty acids are formed, which soon combine with calcium to form calcium soaps. Virchow observed calcification in the form of soaps in a lipoma, and Jaeckle¹ found that a calcifying lipoma contained 29.5 per cent. of its calcium in the form of calcium soaps. Klotz² obtained staining reactions in calcifying tissues that suggested the presence of soaps, which he also extracted by solvents, and he strongly urges, as the first step in the formation of pathological calcified masses, that the calcium is first laid down as soaps, afterward undergoing a transformation into the less soluble phosphate and carbonate. Fischler and Gross³ also obtained microchemical reactions for soaps in the margins of infarcts and in atheromatous areas, but not in caseous areas; they therefore consider that calcium-soap formation is an important step in the process of pathological calcification, but that it is not essential.

On the other hand, Wells,⁴ studying large quantities of material chemically, found but most minute traces of calcium soaps in calcifying matter, even in the earliest stages, and also very small amounts of other soaps or fatty acids, and, therefore, questions the occurrence of calcium soaps as an essential step in calcification, although not doubting that under certain conditions (*e. g.*, calcifying lipomas, fat necrosis) this may occur. In calcification at all stages the proportion of calcium carbonate and phosphate was found quite constant, and exactly the same as in normal bone; namely, in the proportion expressed by the formula $3(\text{Ca}_3(\text{PO}_4)_2) \cdot \text{CaCO}_3$, which Hoppe-Seyler advanced to express the composition of the salts of bone. Hence it seems probable that there are no essential differences between the processes of ossification and pathological calcification, and there seems to be as yet no reason for assuming that in the former calcium soaps constitute an essential step in the process.

Phosphoric Acid in Calcification.—It has generally been assumed that in normal ossification the calcium is combined by phosphoric acid, which probably is derived from the cartilage cells, possibly through autolysis of the nucleoproteids or some similar process. Grandis and Mainini,⁵ by using microchemical

¹ *Zeit. physiol. Chem.*, 1902 (36), 53.

² *Jour. Exper. Med.*, 1905 (7), 633; 1906 (8), 322.

³ *Ziegler's Beitr.*, 1905 (7th suppl.), 339.

⁴ *Loc. cit.*

⁵ *Arch. per la sci. Med. Torino*, 1900 (24), 67.

methods, thought that they found evidence that the phosphorus of ossifying cartilage is converted from an organic combination into an inorganic form (P_2O_5), which then takes up calcium from the blood. The methods used have been questioned, and Pacchioni,¹ from his studies, was inclined to the opinion that the calcium entered the cartilage already combined as phosphate. Wells implanted various tissues that had been killed and sterilized by boiling into the abdominal cavity of rabbits, and found that tissues rich in nucleoproteids showed no tendency to take up calcium in greater amounts than did tissues poor in nucleoprotein, which result speaks against the idea that phosphoric acid derived from nucleic acid combines the calcium. On the other hand, implanted cartilage soon became thoroughly impregnated with calcium salts, which seemed to be deposited in the same proportion as to carbonate and phosphate as in bone.

Physical Absorption of Calcium Salts.—As there could be no question of “vital activity” on the part of this boiled cartilage, it seems most probable that there exists in cartilage a specific absorption affinity for calcium salts, similar to the absorption affinity that Hofmeister² observed exhibited by other organic colloids (gelatin disks) toward various crystalline substances in solution. Pfaundler has also demonstrated that cartilage in the test-tube has a specific absorption affinity for calcium. It is doubtful if ossification can be explained in this simple manner, however, for on this basis we should expect the calcium to be easily washed out of the bones, and an increase in calcium should lead to increased ossification. Furthermore, it does not account for the remarkable specific affinity of cartilage for calcium salts.

OSTEOMALACIA³

In this condition the quantity of inorganic salts in the bone is greatly decreased, while, at the same time, their place is taken in part by new-formed osteoid tissue; as a result, the proportion of the weight of the bone formed by inorganic salts is reduced to as low as 20 to 40 per cent., instead of being from 56 to 60 per cent., as in normal bone.⁴ This suggests that the cause of the disease may be a solution of the lime salts by some acid, in support of which Schmidt has reported the finding of lactic acid in the altered substance of the bones in osteomalacia,

¹ *Jahrb. f. Kinderheilk.*, 1902 (56), 327.

² *Arch. exper. Path. u. Pharm.*, 1891 (28), 210.

³ See also review in Albu and Neuberg's “*Mineralstoffwechsel*,” Berlin, 1906, pp. 124–127.

⁴ Full figures given by Senator, *Ziemssen's Handbuch*, 1879 (13), 236.

while other observers have stated that in osteomalacia the alkalinity of the blood is reduced, and that lactic acid appears in the urine. All the above statements are of questionable value, and it is improbable that there is any such degree of acidity or, better, lack of alkalinity, in the blood or fluids in the bones as to dissolve out the inorganic salts. Levy¹ found that in osteomalacia the proportion of calcium carbonate and phosphate in the bones remains constant, as also does the proportion of calcium and phosphoric acid; if the decalcification occurred through solution by lactic or other acids, the carbonate should be decomposed first, whereas the lime salts seem to be taken out as molecules of calcium carbonate-phosphate; *i. e.*, in the same proportion as they exist in the bone. On the other hand, it has been found in Pawlow's laboratory that dogs kept for long periods after a pancreatic fistula has been established, develop a condition resembling osteomalacia,² which would seem most reasonably explained as due to the constant loss of alkali in the pancreatic juice. Histologically, absorption seems to depend largely upon a direct eating out of bone tissue, both organic and inorganic substance, by osteoclasts (Cohnheim), followed by a formation of an uncalcified osteoid tissue. (Senile osteoporosis differs chiefly in that no new osteoid tissue is formed.) According to Schmidt and to Langendorff and Mommsen, this new-formed osteoid tissue yields no gelatin, and, therefore, is quite different from normal osteoid tissue. It is not established, however, that this alteration is a constant occurrence in osteomalacia. In many cases of osteomalacia the rapid rate of progress of the disease indicates that it is not simply a normal absorption of lime salts with defective replacement, but that an excessive absorption must occur (Pommer³). Chabrie⁴ found that much of the calcium absorbed is replaced by magnesium, so that the latter may be in excess of the former; he found in one case 22.2 per cent. of CaO and 26.9 per cent. of MgO. Malcolm⁵ has found that ingestion of considerable quantities of magnesium salts causes loss of calcium in adult animals, and hinders its deposition in growing animals, but there is no evidence to connect this fact with the increased magnesium in the bones in osteomalacia.

Studies of metabolism in osteomalacia have shown a loss of

¹ Zeit. physiol. Chem., 1894 (19), 239.

² Personal communication from Dr. Boris Babkin.

³ Vierordt, Nothnagel's System, vol. 7, part ii, p. 124.

⁴ Les phenomenes chim. de l'ossification, Paris, 1895.

⁵ Jour. of Physiol., 1905 (32), 182.

calcium by the body, as shown by the following table given by Goldthwait *et al.*:¹

	Limbeck.	Neumann.	Goldthwait.
CaO in urine (gm.)	1.773		3.859
CaO in feces	3.834		1.800
Total excreted	5.607	11.65	5.66
Total in food	2.965	11.26	4.56
Loss of CaO	2.965	0.39	1.10

These authors also found a considerable retention of nitrogen and sulphur, which they suggest may be retained in the new-formed osteoid tissue; magnesium is also retained, probably being substituted for calcium in the bones.

Castration of women with osteomalacia has been frequently, but not always, followed by improvement or recovery, and Neumann, and also Goldthwait, have found that in these cases the calcium loss is replaced by a marked calcium retention after the operation. What the relation of the ovaries to calcium metabolism or to osteomalacia may be has not yet been ascertained. Scharfe² and Bulins³ both state that there are no characteristic or constant structural alterations in the ovaries in osteomalacia. McCrudden⁴ found that the improvement in calcium metabolism observed after castration may be but temporary, and therefore believes that the primary cause of the disease does not lie in the ovaries.

RICKETS⁵

As with osteomalacia, chemical studies of the bones in rickets have thrown little light upon the etiology or pathogenesis of this condition. As the following table (taken from Vierordt⁶) shows, there is a marked deficiency in the proportion of inorganic salts in the bones in rickets. The proportion of the different salts, seems to be quite the same as in normal bone.

¹ Goldthwait, Painter, Osgood and McCrudden, *Amer. Jour. Physiol.*, 1905 (14), 389.

² *Cent. f. Gyn.*, 1900 (24), 1216.

³ *Beitr. z. Geb. u. Gyn.*, vol. 1.

⁴ *Amer. Jour. of Physiol.*, 1906 (17), 211.

⁵ Complete literature and full discussion by Pfaundler, *Jahr. f. Kinderheilk.*, 1904 (60), 123; also see Albu and Neuberg, "Mineralstoffwechsel," Berlin, 1906, pp. 119-124.

⁶ Nothnagel's System, vol. 7, part ii, p. 21.

	Normal bone of a two months' old child.			Rachitic bones.			
	Tibia.	Ulna.	Femur.	Tibia.	Humerus.	Ribs.	Vertebrae.
Inorganic matter	65.32	64.07	20.60	33.64	18.88	37.19	32.29
Organic substance	34.68	35.93	79.40	66.36	81.12	62.91	67.71
Calcium phosphate	57.54	56.35	14.78	26.94 }	15.60		
Magnesium phosphate . . .	1.03	1.00	0.80	0.81 }			
Calcium carbonate	6.02	6.07	3.00	4.88	2.66		
Soluble salts	0.73	1.65	1.02	1.08	0.62		
Collagen (or ossein)	33.86	34.92	72.20	60.14 }	81.22		
Fat	0.82	1.01	7.20	6.22 }			

As an essential difference from osteomalacia is the fact that in rickets there is a failure on the part of the osteoid tissues to calcify, whereas in osteomalacia absorption of calcified tissue takes place with subsequent substitution by osteoid tissue. Furthermore, in rickets the deficiency in calcium is only present in the bones, whereas in osteomalacia the soft tissues are also poor in lime salts.

None of the various hypotheses as yet advanced to explain this defective ossification has satisfactorily explained all the observed facts. That a deficiency of calcium in the food is the cause of rickets is a most natural assumption, but it has not been proved that this is the case. Young animals fed on calcium-poor foods show, naturally enough, defective development of the bone, but this differs essentially from rickets in that the bone formed is defective chiefly in amount rather than in quality (Stöltzner). Furthermore, such "pseudo-rachitic bone" possesses a marked affinity for calcium salts, and takes them up as soon as they are supplied (Pfaundler). Bland-Sutton, Cheadle,¹ and others consider that a deficiency of fat and proteid in the diet is the essential cause. Zweifel and others have advanced the idea that there is a defective absorption of calcium from the foods, depending upon a lack of HCl in the gastric juice; this hypothesis seems to be poorly founded. In view of the fact that rickets is not solely a disease of bone tissue, but that all the various important viscera, as well as the muscles and tendons, show pathological changes, it seems most reasonable that rickets should be looked upon as a *constitutional* disease, in which the bone changes are prominent chiefly because the disease occurs at a time when the bone tissue is most actively forming and when the other organs are relatively quite completely

¹ Allbutt's System, 1897 (3), 108.

developed. Stöltzner,¹ finding evidence that rickets does not depend upon either lack of calcium in the food or deficient absorption of calcium, and that the blood in rickets is of normal alkalinity, looks upon the failure of calcification as depending upon an abnormality in the calcified bone tissue itself. He finds evidence of a preliminary alteration in normal osteoid tissue which prepares it to take the salts out of the blood, and Pfaundler² supports this view, suggesting that this preparatory change in the osteoid tissue may depend upon autolysis, which is perhaps deficient in rickets.³

CONCRETIONS

All pathological concretions appear to be laid down according to a definite law. There must first be a *nucleus* of some substance different from the substance that is to be deposited, and which is most frequently a mass of desquamated cells, but may consist of clumped bacteria, masses of mucus, precipitated proteids, or a foreign body of almost any sort. Upon this nucleus substances crystallize out of solution, much as cane-sugar crystallizes on a string to form rock candy, but with the important exception that among the crystals is usually deposited more or less mucin or other organic substance, which forms a framework in which the crystals lie, and which remains, if the crystals are dissolved out, as a more or less perfect skeleton of the concretion. In no case would the concretion form were it not that the solution is overcharged with some substance, but not infrequently it is the presence of the nucleus that leads to the precipitation of the substance; *i. e.*, the nucleus may play either a primary or a secondary rôle. With few exceptions, the dissolved substance is deposited in crystalline form, although the crystalline structure may in time partly disappear through condensation or through filling of the interstices with some other material. Even so structureless a substance as amyloid may, when forming concretions, appear in a crystalline form (Ophüls). The structure of a concretion depends upon two factors: The crystals tend to be deposited at right angles to the surface, and thus give a *radiating* structure; but the rate of deposition is usually irregular, and during the periods of quiescence the surface tends to become covered with mucin or other organic substances, hence we also get a *concentric, laminated*

¹ Jahrb. f. Kinderheilk., 1899 (50), 268.

² *Loc. cit.*

³ See also Nathan, Med. News, 1904 (84), 391.

structure. Frequently both of these lines of formation are easily discerned, but either one or the other may become obscured.

The chemistry of concretions is, therefore, a relatively simple matter, and it remains merely to give the chief facts concerning the formation and composition of the different varieties.

BILIARY CALCULI

As may be judged from the above statements, concretions are never composed of one substance in a pure form, but usually consist of a mixture of the constituents of the fluid in which they are developed. This is particularly true of gall-stones, which contain in greater or less quantities several or all of the constituents of the bile. While cholesterin forms the greater part of nearly all biliary concretions, and is present in greater or less amounts in all, calcium salts of the bile-pigments are always present; usually inorganic salts of calcium (carbonate and phosphate) are also present, as well as small amounts of fats, soaps, lecithin, mucus, and other products, and occasionally traces of copper, iron, and manganese.¹ The quantity of bile salts, the chief constituent of the bile, is usually extremely minute, apparently only so much as may percolate into the crevices of the concretion. However many stones there may be in a gall-bladder, they usually are all of approximately the same composition and structure.

In gall-stones from the domestic animals the proportion of inorganic salts is usually much higher than it is in man.

Naunyn has classified gall-stones according to their composition, as follows:

1. "**Pure**" **Cholesterin** ² **Stones**.—The purity is only relative, since even the purest always contain some pigment as well as a stroma and a nucleus; but the amount of cholesterin may reach 98 per cent., and is usually over 90 per cent. Crystalline structure is usually well marked, while stratification is slight. The color varies from nearly pure white to yellow, or even brown on the surface.

2. **Laminated Cholesterin Stones**.—These consist of about 75–90 per cent. of cholesterin, and differ from the preceding form in containing more pigment, which is deposited in layers alternating with the white layers of cholesterin. The pigment

¹ Gall-stones have been found enclosing droplets of mercury. (Naunyn, Frerichs.)

² Concerning composition and occurrence of cholesterin, see pages 28 and 346.

here, as in all other gall-stones, consists always of the calcium salts of the pigments—not of pure bilirubin and biliverdin themselves. Considerable calcium carbonate is also usually present, particularly in the green layers of biliverdin calcium.

3. **Common Gall-bladder Stones.**—The composition of this form is but little different from the above, the chief difference being in the structure. They present externally a firmer crust, usually distinctly laminated; in the center is a softer pigmented nucleus which frequently shows a central cavity containing fluid. Such calculi are not distinctly crystalline in structure, and are small, seldom larger than a cherry.

4. **Mixed Bilirubin-calcium Calculi.**—These generally occur singly, but sometimes in groups of three or four, and are of large size. Although the chief constituent is bilirubin-calcium, there is always much cholesterin, often over 25 per cent. Copper and traces of iron may also be present. Their structure is laminated, with sometimes a crystalline cholesterin nucleus.

5. **“Pure” Bilirubin-calcium Calculi.**—In addition to the chief constituent, *biliverdin-calcium*, *bilifuscin*, and *bilihumin*¹ are practically always present. *Bilihumin* is at times the chief ingredient, and may form over half of the substance; *bilicyanin* is rarely present. There is always some cholesterin, but sometimes only traces. These calculi are small, from the size of a grain of sand to that of a pea, and they occur in two distinct forms. One form is of wax-like consistence; the other is harder, steel-gray or black in color, with a metallic luster. Pure bilirubin and biliverdin, not combined with calcium, are practically never present in concretions.

6. **Rarer Forms.**—(a) *Amorphous and incompletely crystalline cholesterin gravel.* Cholesterin externally giving them a pearly luster; pigment in the center.

(b) *Calcareous Stones.*—Consist chiefly of a mixture of calcium carbonate and bilirubin-calcium. Calcium carbonate may occur either as a superficial crust, or as small masses within an ordinary calculus; calcium sulphate and phosphate occur rarely in traces. Stones consisting mainly of calcium carbonate are extremely rare in man, but more frequent in cattle and other

¹ *Biliverdin* differs from *bilirubin* in containing one more atom of oxygen in the molecule, and it is easily formed from bilirubin—even exposure to air will slowly bring about the oxidation. *Bilifuscin* is a still more oxidized derivative—so much so that it does not give Gmelin's reaction (with $\text{HNO}_3 + \text{HNO}_2$) for bile-pigments. *Bilihumin* represents the most oxidized of these products, is brown in color, and is the chief constituent of the residue left after treating gall-stones with ether, alcohol, and chloroform to dissolve out the cholesterin.

herbivora, in which all forms of concretions contain much calcium, either combined with pigment or as carbonate and phosphate.

(c) *Concretions with included bodies, and conglomerate stones.*

(d) *Casts of Bile-ducts.*—Occur particularly in cattle, and consist chiefly of bilirubin-calcium. Rarely and imperfectly formed in man.

Formation of Gall-stones.—We owe our present understanding of the chemistry and pathology of the formation of gall-stones chiefly to Naunyn¹ and his pupils. Former observers, having learned that bile normally contains cholesterin (Hammarsten found from 0.06–0.16 per cent. in human bile), sought the cause of gall-stones in either an increased elimination of cholesterin by the liver, or a decrease in the power of the bile to hold the cholesterin in solution. Thus Frerichs, finding that the presence of large amounts of bile salts and an alkaline reaction favored the solution of cholesterin, imagined that a diminution of either bile salts or alkalinity led to the precipitation of the cholesterin. Naunyn and his pupils, however, demonstrated that the amount of cholesterin present in the bile does not depend upon the amount taken in the food or the amount present in the blood; and that it did not vary in disease, except when gall-stones were present. They concluded that the cholesterin of the bile is neither a product of general metabolism nor a specific secretion-product of the liver. Finding that pus and the secretions from inflamed mucous membranes (bronchitis) contained as much cholesterin as did normal bile, and often more, they concluded that the chief source of cholesterin in gall-stone formation was from the degenerating and desquamated epithelial cells of the gall-bladder and bile tracts. This idea was supported by the large amount of cholesterin found in the contents of gall-bladders shut off from the common duct, and by the formation of gall-stones in such isolated gall-bladders. Further evidence has since been brought forward in favor of this same view,² until it is now generally accepted as correct. It is now believed that the ordinary steps in the formation of a cholesterin concretion are as follows: Some injury to the mucous membrane of the bile tracts is the

¹ An English translation of this classic work, by A. E. Garrod, has been published by the Sydenham Society, 1896, vol. 158.

² Thus Wakeman (quoted by Herter, Trans. Congress Amer. Physicians, 1903 (6), 158; excellent résumé) was able to cause an increase in the cholesterin of the bile in the gall-bladder of dogs by injecting into it HgCl_2 , phenol, or ricin. At first the cholesterin seems to be contained largely in the degenerating desquamated cells.

starting-point; this injury is usually produced by infection, the colon and typhoid bacilli being the most common organisms in this process.¹ It is probable that injury alone is not sufficient to cause gall-stone formation, but infection is essential (Miyaka²). Through the degeneration of the epithelial cells an excess of cholesterin is formed, while at the same time the desquamated cells and clumped bacteria offer suitable nuclei upon which the cholesterin begins to crystallize out. Apparently after the calculi have reached a certain size they cause sufficient mechanical injury to keep up the cell degeneration and cholesterin formation, even after the infection has subsided. A certain amount of infection and inflammation is a favoring condition, however, for Harley and Barratt³ found that fragments of cholesterin calculi introduced aseptically into the gall-bladders of dogs were slowly dissolved and disappeared, but this was prevented by infecting the gall-bladder with *B. coli*. According to Naunyn's investigations, it is not an alteration in the composition of the bile, as formed in the liver, which causes the precipitation of cholesterin, but rather the presence of the nidus, and the production of large quantities of cholesterin in immediate proximity to this nidus, that determines the formation of a concretion. In case the bile stagnates in the gall-bladder, the cholesterin that is being constantly formed by the normal disintegration of surface epithelium accumulates, until, even without infection, there forms a sediment of soft yellowish and brownish masses, consisting chiefly of cholesterin and bilirubin-calcium. From this material calculi may eventually form, and by their irritation lead to further formation of cholesterin and increased growth. But bacteriological studies indicate that generally an infectious influence is present in cholelithiasis, and bacilli may be found alive in gall-stones for remarkably long periods.

It was formerly supposed that the calcium-pigment concretions were produced by the presence of excessive calcium in the bile, derived particularly from lime-laden drinking-water, but it has been demonstrated that increase of calcium in the food does not cause an increase in the amount in the bile. Furthermore, on concentrating bile, which contains both bilirubin and calcium, the free bilirubin separates out and not the calcium

¹ See Cushing (Johns Hopkins Hosp. Bull., 1899 (10), 166), who produced gall-stones experimentally by injecting typhoid bacilli into the circulation after injuring the gall-bladder. Literature on the relation of bacteria to gall-stones given by Cushing; also by Pratt, Amer. Jour. Med. Sci., 1901 (122), 584; by Bierring, Jour. Amer. Med. Assoc., 1904 (43), 1099; and by Herter (*loc. cit.*).

² Mitt. a. d. Grenzgeb. Med. u. Chir., 1900 (6), 479.

³ Jour. of Physiol., 1903 (29), 341.

compound of bilirubin; and also Naunyn found that the bile salts prevent precipitation of calcium-bilirubin, even when calcium salts are added in considerable amounts. Apparently it is the presence of proteid substances that leads to the precipitation of this compound from bile, and hence the formation of pigment calculi is also favored or initiated by inflammation of the bile tracts, particularly as most of the calcium salts seem to come from the mucous membrane;¹ later, as we have seen, these pigment concretions often become covered with cholesterin derived from the injured epithelium, and the common mixed calculi are then formed. In view of the fact that much of the pigment in these calculi is composed of the oxidation products of bilirubin, especially *bilihummin*, it is possible that oxidation processes in the stagnating bile are important causes of the precipitation; Naunyn suggests that bacteria may be the cause of the oxidation. Pigment calculi are particularly important as the starting-point of the larger mixed calculi. It is possible, Naunyn believes, for the pigment to be later gradually replaced by cholesterin.

URINARY CALCULI²

These differ from the bile concretions in two important respects: first, their constituents are derived from the secretion of the kidney rather than from the walls of the excretory passages, and they are usually deposited on account of an oversaturation of the urine, or on account of a change in composition of the urine, which renders them insoluble. Second, the composition of urinary calculi is usually less mixed than that of biliary calculi, although seldom, if ever, is it pure. Thus, Finsterer found but six concretions composed of only one substance, in a collection of 114 calculi. As with the bile, the chief constituent of the urine (urea) is so soluble that it never forms concretions, but only the less soluble minor constituents are thrown down. For the formation of calculi, however, it is not sufficient to have merely an excess of a substance in the urine, for we may have deposition of urates, phosphates, or uric acid in simple crystalline form without the formation of calculi. A *nucleus* of some sort must be present as well as a *binding substance*, which is often mucus derived from the walls of the

¹ Baldwin (quoted by Herter, *loc. cit.*) found human cystic bile to contain an average of 0.072 per cent. of calcium, the amount not showing any constant relation to the quantity of cholesterin present. The presence of calcium pigment stones was not associated with an excess of calcium in the bile.

² General bibliography given by Finsterer, *Deut. Zeit. klin. Chir.*, 1906 (80), 414.

passages, although the center of the concretion most often consists of uric acid or urates. Infection does not always play so important a part here as it seems to in the formation of biliary calculi; calculi formed because of changes in the urinary composition independent of infection are often called "primary," in contradistinction to those arising from changes in composition brought about by infection and ammoniacal decomposition. Because of the injury produced by a primary calculus, infection frequently results, and then the primary calculus may become the nucleus of a secondary calculus; indeed, on account of the change of reaction, the primary calculus may be dissolved out, and its place taken by the secondary deposit (*metamorphosed calculi*). In structure urinary calculi usually show both radiating and concentric lines of formation, and when the chief constituents are dissolved away, an organic framework remains. They are generally classified according to their composition, as follows:

Uric-acid Calculi.—Uric acid is but slightly soluble, only one part dissolving in 39,480 of pure water at 18°, and it is even less soluble in the presence of acids. The presence of sodium diphosphate in the solution makes it much more soluble, and various organic bodies also favor its solution, among them being the urinary pigments. As can be seen, the maintenance of uric acid in solution is by a small margin, even in normal conditions; hence the mere cooling of the urine frequently suffices to cause an abundant deposition of uric acid combined with pigment, as the familiar "brick-dust" deposit. The formation of uric-acid calculi is, therefore, not only a question of the amount of uric acid in the urine, but depends even more upon the amount of the substances that hold it in solution, and as both these factors are subject to wide variations under both physiological and pathological conditions, uric-acid and urate calculi are the commonest of urinary concretions. Uric acid is eliminated combined chiefly with sodium, potassium, and ammonium; according to some authors, as a biurate, according to others, as a quadriurate. If the urine is excessively acid, it contains much acid phosphates, which withdraw part of the bases from the uric acid, and this, when free, crystallizes out if in excess. Hence the formation of uric-acid concretions is favored by high acidity of the urine, by concentration of the urine, or by an increased elimination of the uric acid. The last may result from excessive nuclein-rich food, or from excessive katabolism of the tissue nucleoproteids (*e. g.*, leucocytosis from inflammatory diseases or leukemia), which conditions are also

usually associated with an increased urinary acidity. (The chemistry of uric acid is discussed more fully in the chapter on "Gout," Chap. xxi.)

Uric-acid calculi are formed chiefly in the pelvis of the kidney, but many pass into the bladder. They are quite hard, and yellow or reddish-yellow in color, because of the presence of *urochrome* and *urobilin*, the former of which seems to be chemically combined and the latter but physically, since it can be washed out with water. *Uroerythrin* or *uromelanin* (a decomposition product of urochrome) may also be present. Not infrequently calcium oxalate is present, sometimes in considerable quantities. Other urinary constituents may be present in small amounts. In case the calculus enters the urinary bladder it may set up irritation leading to infection; the urine then becoming alkaline, calcium and ammonio-magnesium phosphate will be deposited upon the surface, and the uric acid will be more or less dissolved out and replaced by the phosphates (metamorphosis).

Urate calculi occur chiefly in new-born or young infants, and rarely in adults. In the young they are related to, and may originate in, the deposits of urates in the pyramids of the kidney (the so-called urate or uric-acid "infarcts"), which have been supposed to result from the decomposition of the nucleoproteids of the nucleated fetal red corpuscles. (See "Uric Acid," Chap. xxi.) The concretions are composed chiefly of either ammonium or sodium urate, but potassium and even calcium and magnesium urate may be admixed. Their genesis in the young probably depends upon injury to epithelium by the excessive urates of the "infarcts," which affords a suitable nucleus for their start; their growth depends chiefly upon the concentration of the infant's urine. In adults they may arise secondary to an ammoniacal decomposition of the urine. Urate concretions are not common; they are generally rather soft, and often much colored by pigments.

Calcium oxalate calculi are the hardest of all concretions (except some forms of the rare calcium carbonate calculi) and in frequency stand next to the uric-acid calculi. Often they show admixtures of urates or uric acid, which latter frequently constitutes the nucleus, and when urinary infection occurs they may in turn serve as the nucleus to phosphatic deposits. On account of the hardness and roughness of these stones they frequently cause bleeding, which may result in their being very dark in color and containing blood-pigment. They are usually first formed in the pelvis of the kidney, and arise chiefly in

persons excreting excessive quantities of oxalic acid. Normally but about 0.02–0.05 gram of oxalic acid is eliminated daily in the urine, apparently all as calcium oxalate, which is kept in solution by the acid phosphates. The amount may be increased by certain foods rich in oxalates, particularly rhubarb, grapes, spinach, etc.; also probably by gastric fermentation.¹ Oxalic acid seems normally to be formed from uric acid, and perhaps also from the carbohydrate group of proteids,² and it is possible that abnormally large amounts arise from these sources under pathological conditions.

Phosphate calculi are formed as a result of decomposition of the urine, with formation of ammonia from the urea. In the ammoniacal solution thus formed the magnesium is precipitated as NH_4MgPO_4 , the calcium as $\text{Ca}_3(\text{PO}_4)_2$, and calcium oxalate and ammonium urate are also thrown down, so that the concretions consist of a mixture of these substances, the magnesium salt being the most abundant. In none does one substance occur in a pure state. Pigments of various kinds, and more or less mucus or other organic constituents of the framework are also present. Phosphate calculi are the typical “secondary” concretions, and they are formed usually in the bladder as a consequence of cystitis, but may be formed in the renal pelvis or in the urethra. In some cases the salts are precipitated in such large quantities that they form great masses of a sediment which does not aggregate into concretions. Occasionally stones consisting principally of $\text{Ca}_3(\text{PO}_4)_2$ or CaHPO_4 are formed, but these are rarities. As the calcium taken in the food is chiefly eliminated in the feces, the amount in the urine does not vary directly with the amount in the food, and the formation of phosphatic concretions is always a matter of urinary reaction and not of diet.³ As these stones fuse to a black, enamel-like mass under the blow-pipe, they have been called “fusible calculi.”

Calcium carbonate calculi are formed frequently in herbivora, but they are very rare in the urinary passages of man, although occurring elsewhere in the body not infrequently. Occasionally these are soft and chalky, but if well crystallized, they are the hardest of concretions.

¹ Baldwin, Jour. Exp. Med., 1900 (5), 27.

² See Austin, Boston Med. and Surg. Journal, 1901 (145), 181.

³ Under the name “struvit stone,” Pommer (Verh. deut. Path. Gesell., 1905 (9), 28) describes a urinary calculus composed of very pure ammonio-magnesium phosphate, forming the hard, rhombic crystals known to mineralogists as “struvit.” This is an example of a phosphate stone formed independent of ammoniacal decomposition, a rare occurrence.

Cystin calculi are rare but very interesting formations. Cystin $\begin{array}{c} \text{S-CH(NH}_2\text{)-COOH} \\ | \\ \text{S-CH(NH}_2\text{)-COOH} \end{array}$ is important as the sulphur-containing portion of the proteid molecule. Under normal conditions all the cystin taken in food is completely oxidized and none (or uncertain traces) appears in the urine. In certain individuals the urine contains considerable quantities of cystin constantly (*cystinuria*, see Chap. xix), and occasionally in these cases soft concretions of nearly pure cystin are formed in the urinary passages. Cystin calculi may reach the size of a hen's egg, are crystalline in structure, and in the urine of such patients the characteristic hexagonal crystals may usually be found. Loewy and Neuberg¹ have contended that the cystin found in urinary calculi is an isomer of the cystin found in the proteid molecule, and that cystinuric patients can completely oxidize proteid cystin, but not the stone cystin. This claim has not been substantiated, however.²

Xanthin Calculi.—Xanthin is the most abundant of the purin bases normally present in urine, but the total amount is extremely small. Like uric acid, it fluctuates in amount according to the amount of destruction of nucleoproteids, either of the food or of the tissues. Concretions consisting chiefly of xanthin, which is often mixed with uric acid, are extremely rare, but a few isolated specimens having been described.

Indigo calculi, derived from the indican of the urine through oxidation, have also been described a few times.

Urostealith calculi, composed of fatty matter, have been occasionally observed. Although some of the concretions described under this head have really represented foreign bodies introduced through the urethra (*e. g.*, Kruckenberg's concretion of paraffin from a bougie), yet true fat concretions do occur. The origin of the fat in these stealiths is unknown, possibly it comes from degenerated epithelium. Horbaczewski³ analyzed such a specimen which had the following percentage composition :

Water	2.5
Inorganic matter	0.8
Organic matter (chiefly proteid)	11.7
Fatty acids	51.5
Neutral fat	33.5
Cholesterin	traces

¹ Zeit. physiol. Chem., 1904 (43), 338.

² Rothera, Jour. of Physiol., 1905 (32), 175. Literature concerning cystin, see Friedmann, Ergeb. der Physiol., 1902 (i), 15; Marriott and Wolf, Am. Jour. Med. Sci., 1906 (131), 197.

³ Zeit. physiol. Chem., 1894 (18), 335.

The fatty acids consisted of stearic, palmitic, and probably myristic acid.

Cholesterin calculi have been found in the urinary bladder in a few instances, the cause being unknown. Horbaczewski¹ describes one weighing 25.4 grams, found in a patient who had previously had cystin calculi; it contained 95.87 per cent. of cholesterin and but 0.55 per cent. of inorganic material. Gall stones have been known to enter the urinary bladder through a fistula between the gall-bladder and urinary bladder.²

Fibrin "calculi," formed from blood-clots, often more or less impregnated with urinary salts, have occasionally been observed.³

General Properties of Urinary Concretions.—The **hardness** depends upon the chemical composition of the calculus. Those composed of amorphous phosphates are the softest; next come those with some admixture of crystalline phosphates. Urate concretions are harder than these, but are still softer than the uric acid and crystalline phosphate calculi. Oxalates are the hardest, except for the rare crystallized calcium carbonate stones. Cystin and amorphous concretions can be scratched with the finger-nail, while even the hardest varieties of calculi can be scratched with a wire nail. Genersich⁴ gives the following degrees of hardness for different calculi: Cholesterin, 1.5–1.6; ammonium urate, 2.5; soft phosphate (Mg), 2.6; hard phosphate (Ca), 2.75; uric-acid stones (also salivary and prostatic calculi, atheromatous patches, and phleboliths, 2.9; calcium oxalate (also rhinoliths and lung stones), 3.3–3.5; calcium carbonate stones of herbivora, 4.5.

The **rate of growth** also varies according to composition, but is, of course, much modified by other factors. Oxalate and urate stones grow most slowly, phosphate stones most rapidly. A urate stone has been known to increase by about two ounces during seven and one half years, while a catheter fragment or other foreign body may become covered with a crust several millimeters thick in a few weeks.⁵

Spontaneous disintegration of urinary concretions is limited almost solely to calculi composed entirely or largely of uric

¹ *Loc. cit.*

² See Finsterer, *Deut. Zeit. klin. Chir.*, 1906 (80), 426.

³ Systems for procedure in determining the nature of urinary calculi are given by Hammarsten (*Text-book of Physiol. Chem.*) and by Smith (*Reference Handbook of Med. Sci.*, 1901 (3), 236).

⁴ *Virchow's Arch.*, 1893 (131), 185.

⁵ Zuckerkandl, *Nothnagel's System*, vol. 19, pt. 2, p. 229.

acid. Out of 121 cases collected by Englisch,¹ in all but 7 this was the case, these being composed of calcium and magnesium phosphate (5), or calcium phosphate or carbonate (1 each). The disintegration is brought about through solution of the binding substance and mechanical shattering of the stone into fragments.

CORPORA AMYLACEA²

In the case of these widely-spread concentric bodies we find the name misleading, for the bodies are not a form of animal starch, as was suggested by their laminated structure and iodine reaction, nor are they so closely related to amyloid material as the name implies. Different authors disagree decidedly concerning the staining reactions of these bodies, but it may be said that the reactions are extremely inconstant. Sometimes the corpora are stained bluish or green with iodine, sometimes brown, often little at all; occasionally they react partly with methyl-violet, but more often they do not; sometimes portions of one body react one way, while the remainder behaves differently. Seldom if ever do the ordinary concretions of the prostate give all the amyloid reactions characteristically, and the same applies to the corpora amylacea of the lungs. It seems improbable that these bodies, which occur in the prostate of every adult (Posner), can be the same as the amyloid, which is seldom observed except as the result of serious processes of tissue destruction. According to their structure they obey the usual laws of the formation of concretions, having a central nucleus and a structural framework of different composition from the chief substance. It seems most probable that they should be interpreted as simple concretions of proteid nature, which form under certain conditions when a nucleus of some sort (usually pigment, degenerated cells, or inorganic crystals) exists in a stagnating, proteid-rich fluid. At times the resulting concretion may be of such a physical nature that it absorbs iodine readily (just as they often show a marked absorption-affinity for pigments), and occasionally it may react metachromatically with methyl-violet, possibly because of the presence of chondroitin-sulphuric acid derived from the mucin of the cavities where the concretions form, but perhaps for some other unknown reasons. Occasionally pure amyloid may form in the tissues typically concentric (or even crystalline) bodies, as in Ophül's case,

¹ Arch. klin. Chir., 1905 (76), 961 (elaborate review).

² General literature, Posner, Zeit. klin. Med., 1889 (16), 144; Lubarsch, Ergeb. allg. Pathol., 1894 (1₂), 180; Ophüls, Jour. Exp. Med., 1900 (5), 111.

but this is the exception. It seems probable that corpora amylacea are usually proteid concretions,¹ and neither amyloid nor animal starch.

The small amount of material available prevents an accurate analysis of the corpora amylacea; it is known that they are very insoluble in water, acids, alkalies, etc., behaving like coagulated proteid in this respect. Even hot concentrated nitric acid will not dissolve them, according to Posner. This author considers lecithin and cholesterin to be important constituents, which view does not seem to have been confirmed. The corpora amylacea of the lateral ventricles seem to consist chiefly of calcium salts deposited in a concentric arrangement through the medium of an organic basis. Posner considers that the presence of lecithin in prostatic corpora prevents their calcification, although this change occasionally does occur.

OTHER, LESS COMMON CONCRETIONS

Pancreatic Calculi.—The cause of the formation of stones in the pancreatic duct is not definitely known, but apparently infection is the most important factor, since simple experimental stasis will not cause their formation.² The calculi consist usually of a mixture of calcium phosphate and carbonate, associated with more or less organic matter, including frequently cholesterin, but all the usual products of proteolysis may be present because of the presence of trypsin. Occasionally the calculi consist chiefly of calcium carbonate, which may be almost pure. Shattock³ has observed a pancreatic concretion composed of calcium oxalate. Sodium phosphate and chloride, magnesium phosphate, and proteids have also been found in these concretions.

Baldoni⁴ found, on analysis of a stone weighing 3.1 grams, the following percentage composition :

Water	3.44
Ash	12.67
Proteids	3.49
Free fatty acids	13.39
Neutral fatty acids	12.40
Cholesterin	7.69
Pigments and soap	40.91
Undetermined	6.01

¹ Ramsden's observations (Proc. Royal Soc., 1903 (72), 156) on the precipitation of proteids by the action of surface contact may have some bearing on the formation of such proteid concretions.

² See Lazarus, Zeit. klin. Med., 1904 (51), 530. Literature.

³ Brit. Med. Jour., 1896 (i), 1034.

⁴ Schmidt's Jahrb., 1900 (268), 210.

Usually, however, pancreas stones consist chiefly of inorganic substances. Johnson and Wollaston report analyses of two stones, one containing 72.30 per cent. calcium phosphate and but 8.80 per cent. organic matter; the other 91.65 per cent. calcium carbonate, 4.15 per cent. magnesium carbonate, and but 3 per cent. organic matter. Legrand¹ found only 0.7 per cent. organic matter in another concretion which contained 93.1 per cent. calcium carbonate. Pancreatic juice, being strongly alkaline, can hold but a small quantity of calcium salts in solution (normally but 0.22 part per thousand—C. Schmidt); presumably the little normally present is held in the form of a colloidal suspension by the proteids. Possibly when stasis occurs, digestion of the proteids leads to the precipitation of the calcium salts, or, more probably, the excessive calcium is largely derived from the exudate from the inflamed ducts, as seems to be the case with the calcium of biliary calculi.

Salivary Calculi.²—These have a similar composition, in the main, to the concretions of the pancreatic duct, except that they generally contain more organic matter, resembling in this respect the “tartar” of the teeth. Bessanez found in one 81.3 per cent. of calcium carbonate and 4.1 per cent. of calcium phosphate, whereas in another the carbonate was but 2 per cent. and the phosphate 75 per cent. Potties has described a calculus with a central portion composed chiefly of uric acid and a peripheral portion containing 69 per cent. of calcium phosphate and 20.1 per cent. of calcium carbonate. Harlay³ found in one specimen 15.9 per cent. organic matter, 75.3 per cent. calcium phosphate, 6.1 per cent. calcium carbonate. Roberg believes that bacteria alone do not usually cause salivary calculi to form, but that a foreign body entering the duct is the chief factor. Increased alkalinity may also favor precipitation of calcium from the saliva. In Roberg’s case of sialolithiasis the saliva was of normal composition.

Intestinal Concretions.—These always have a nucleus of some indigestible foreign substance, most often hair, but sometimes cellulose structures or solid indigestible particles, including gall-stones, fruit-stones, bone, etc. The bulk of the concretions is usually made up chiefly of ammonio-magnesium phosphate, with some calcium phosphate, carbonate, and sulphate, proteid matter, and occasionally calcium and magnesium

¹ Jour. Pharm. et Chim., 1901 (14), 21.

² Literature, see Roberg, Annals of Surgery, 1904 (39), 669.

³ Jour. Pharm. et Chim., 1903 (18), 11.

soaps. Two intestinal concretions analyzed by Schuberg¹ had the following percentage composition when dried :

Ammonio-magnesium phosphate	57.1	63.9
Calcium phosphate	15.7	23.8
Calcium carbonate		4.6
Calcium sulphate	3.0	0.7
Alcohol-ether extract	1.9	0.8
Other organic substances	21.5	6.0

In countries where oatmeal is largely eaten, intestinal concretions are not infrequent ; they contain calcium and magnesium phosphate, about 70 per cent. ; oatmeal bran, 15–18 per cent. ; soaps and fats, about 10 per cent. (Hammarsten). Occasionally concretions consisting largely of fat and soaps are found, and after taking large doses of olive oil masses of solidified oil may be passed that are readily mistaken for softened gall-stones, for the removal of which the oil is usually given.

Bezoar stones are intestinal concretions probably coming from *Capra ægagrus* and *Antelope dorcas*. One variety consists chiefly of *lithofellic acid*, $C_{20}H_{36}O_4$, which is related to cholalic acid, and gives an aromatic odor when heated. The other variety ("false bezoars") does not give the aromatic odor, and consists chiefly of *ellagic acid*, $C_{14}H_6O_8$, a derivative of gallic acid, and, therefore, probably derived from the tannin of the food of the antelopes.

Intestinal "sand" occurs as (1) "false sand," consisting of particles of indigestible food, such as the sclerenchymatous particles in the flesh of pears ; and (2) true sand, consisting largely of inorganic material, and formed, according to Duckworth and Garrod,² in the upper part of the large intestine. Analyses of specimens by Garrod showed the following composition :

Water	12.4	
Organic material	26.29	
Inorganic material	61.31	containing {
		calcium oxide 54.98
		phosphorus pentoxide 42.35
		carbon dioxide 2.20
		traces of Mg, Fe, etc. 0.47

Analyses by other observers have given similar results, the absence of the large proportion of magnesium found in larger concretions being striking.

The color is usually brown, due chiefly to urobilin, unaltered bile-pigments being scanty.

Preputial concretions sometimes form beneath a prepuce that cannot be retracted, through deposition of urinary salts on

¹ Virchow's Arch., 1882 (90), 73.

² Lancet, 1902 (i), 653. Full résumé and literature.

and in the accumulated smegma.¹ The composition is, therefore, very mixed, and consists of an organic base containing much cholesterin, fats, and soaps, incrustated with inorganic substances, of which ammonio-magnesium phosphate and calcium phosphate are usually the most abundant.

Prostatic concretions originate in the corpora amylacea (which have been discussed on page 386) through growth by accretion of inorganic salts, until they may reach considerable size. Stern² gives the following results of analysis of such a prostatic stone:

Water	8.0
Organic matter	15.8
Lime	37.64
Magnesia	2.38
Soda	1.76
Potash	0.5
Phosphoric acid	33.77
Iron	trace

Lung Stones.³—These may be formed in the bronchi, through accretion about an inorganic nucleus, similar to the formation of calculi in other epithelial-lined passages; or they may consist of calcified areas of lung tissue or peribronchial glands, which have been sequestered through suppuration and have entered the bronchi. In the latter case, the calculi present the usual composition of pathological calcified areas. That the expectorated stones frequently represent calcified tubercles is shown by Stern³ and by Bürgi,³ who demonstrated tubercle bacilli in decalcified lung stones. The following percentage figures are taken from Ott⁴:

	Specimen I.	Specimen II.
Calcium phosphate	52.0	72.8
Magnesium phosphate		1.0
Magnesium carbonate	2.0	
Calcium carbonate	13.0	6.0
Fat and cholesterin	24.0	7.0
Other organic substances	4.0	10.0

Rhinoliths⁵ are formed about nasal secretions, blood-clots, and most frequently about foreign bodies. They therefore contain much organic substance in addition to the inorganic

¹ See Zeller, *Arch. klin. Chir.*, 1890 (41), 240.

² *Amer. Jour. Med. Sci.*, 1903 (126), 281.

³ Literature, Poulalion, Thesis, Paris, 1891; Stern, *Deut. med. Woch.*, 1904 (30), 1414. Bürgi (*Deut. med. Woch.*, 1906 (32), 798) has recently described two cases in which the concretions consisted chiefly of calcium phosphates.

⁴ "Chem. Path. der Tuberc.," 1903, p. 92.

⁵ Literature, Scheppegegrell, *Jour. Amer. Med. Assoc.*, 1896 (26), 874; Gerber, *Deut. med. Woch.*, 1892 (18), 1165.

salts deposited upon them. Berlioz¹ gives the following table from the analysis of four specimens :

	1	2	3	4
Weight of specimens, grams	3.75	1.34	0.63	0.95
Water	5.80	5.10	4.00	6.90
Organic matter	16.60	18.20	16.00	18.10
Calcium phosphate	62.02	60.61	61.40	47.63
Magnesium phosphate	5.08	6.28	3.93	6.68
Calcium carbonate	10.50	9.81	14.67	20.69
Traces of iron	Doubtful.	Distinct.	Doubtful.	Distinct.

Tonsillar concretions consist chiefly of carbonate and phosphate of calcium deposited upon the inspissated secretions and desquamated cells of the tonsillar crypts. According to some authors, leptothrix threads frequently form the nucleus of the concretions.

Cutaneous concretions are occasionally observed, located chiefly in the subcutaneous tissue, often occurring multiple. The origin is possibly in dilated sebaceous glands with retained secretions. Unna considers that calcium soaps are formed as a first step, but an analysis of such material by Harlay² showed 87.2 per cent. of ash, 12.8 per cent. organic matter, 0.9 per cent. of fat ; calcium phosphate constituted 65.2 per cent., and calcium carbonate 16.4 per cent. Gascard³ found in similar material 23.4 per cent. organic matter, and of the inorganic matter, 91.1 per cent. was calcium phosphate, and 8.9 per cent. calcium carbonate.

Gouty deposits observed in the subcutaneous tissues, as well as along the tendons, articular cartilages, etc., consist usually of nearly pure biurate of sodium and potassium. Ebstein and Sprague⁴ found the composition of such material to be as follows :

Uric acid	59.70
Tissue organic matter	27.88
Sodium oxide	9.30
Potassium oxide	2.95
Calcium oxide	0.17
MgO, Fe, P ₂ O ₅ , S	traces

After a time, however, calcium salts may be deposited, and Dunin⁵ has observed deposits resembling gouty tophi that were merely calcium salts.

¹ Jour. Pharm. et Chim., 1891 (23), 447.

² Jour. Pharm. et Chim., 1903 (18), 9.

³ *Ibid.*, 1900 (12), 262.

⁴ Virchow's Arch., 1891 (125), 207.

⁵ Mitt. Grenzgeb. Med. u. Chir., 1905 (14), 451.

PNEUMONOKONIOSIS

In a number of cases of the different forms of this condition quantitative analyses have been made, which may be briefly discussed as follows: Not only does the lung of every adult contain considerable amounts of coal-pigment stored up in the connective tissues (and also in the peribronchial glands), but also, which is perhaps less generally appreciated, considerable quantities of silicates are also present (chalicosis) from inhaled dust. Woskressensky¹ found silicates in all of 54 lungs examined, except two from infants. The lungs of individuals whose occupations do not expose them especially to dust inhalation contain increasing amounts of silicates in direct proportion to age; the silicates constitute then from 3.5 to 10 per cent. of the total ash of the lungs. There is always a larger proportion of silicates in the peribronchial glands than in the lungs, constituting from 6 to 36 per cent. of the ash, corresponding with Arnold's observation that in gold-beaters the glands contain more metal than the lungs. In stone-workers Schmidt found a higher proportion of SiO_2 in the lungs than in the glands. In normal adults the amount of coal-pigment is greater than the amount of silicates; in children the reverse is the case.

Thorel² reports that the lungs of a worker in soapstone contained 3.25 per cent. of ash, including 2.43 per cent. of soap-stone.

In *siderosis* iron has been found in the lungs in proportions varying from 0.5 per cent. to 7.9 per cent. of the dry weight, the last amount having been found by Langguth³ in the lungs of an iron miner, which contained also 11.92 per cent. of SiO_2 .

An analysis of a lung from a knife-grinder is reported by Hodenpyl,⁴ which gave the following results: Total weight of dried and powdered lung, 48.1009 grams; total solids, 44.7986; ether-soluble substance, 14.6017. Composition of the ether-soluble substance: free fatty acids, 7.498; neutral fats, 4.044; cholesterin, 3.037. Proteids, 15.4759; charcoal (total carbon less proteid carbon), 7.198; ash, 4.2903. The composition of the ash (in grams) was as follows: K_2O , 0.2167; Na_2O , 0.3523; CaO , 0.0965; Fe_2O_3 , 0.0879; Al_2O_3 , 1.4628; SO_3 , 0.0704; P_2O_5 , 0.9565; SiO_2 , 1.2043. The amount of emery, represented by the oxides of aluminum and silicon made up more than one-half of the ash, and the iron constituted about one-fourth. The man had worked at the trade of knife-grinder for about fifteen years.

¹ Cent. f. Path., 1898 (9), 296.

² Ziegler's Beitr., 1896 (20), 85.

³ Deut. Arch. klin. Med., 1895 (55), 255.

⁴ Medical Record, 1899 (56), 942.

CHAPTER XVI

PATHOLOGICAL PIGMENTATION

MELANIN¹

Melanin occurs normally as the coloring-matter of hair, of the choroid of the eye, of the skin, in the pigment matter of many lower animals, and most strikingly as a defensive substance in the "ink" ejected by squids to render themselves invisible in the water. Pathologically melanin occurs chiefly as the result of an excessive production of this pigment by cells normally forming it, as in freckles, melanotic tumors, and Addison's disease (probably). Cells that do not normally form melanin probably do not acquire this power in pathological conditions. Pathological failure to form melanin is also observed, as in skin formed in the healing of wounds and after syphilitic lesions; or in *albinism*, in which the failure to form melanin may often be attributed to hereditary influences.

Melanin seems always to be produced through metabolic activity of specialized cells. The idea, which was formerly advanced, that it is derived from hemoglobin as a product of disintegration, seems to have failed entirely of substantiation. In malaria we frequently find a diffuse pigmentation of the skin of such a nature as to suggest strongly a melanin formation, and this has been cited as an example of the production of melanin from hemoglobin. Carbone has proved, however, that this malarial pigment is derived from hematin. The amount of iron contained in melanin has been much investigated, as bearing upon the question as to whether the melanin is derived from hemoglobin or not, and the results obtained by the best methods indicate that the amount of iron present is usually extremely small, and often it is entirely absent; furthermore, the presence of iron is no proof that the pigment is derived from hemoglobin, since many other proteids contain iron.

Composition of Melanin.—The elementary composition of different specimens of melanin examined by various observers has been found to vary greatly. This probably depends on

¹ Literature and résumé given by v. Fürth, *Cent. f. Pathol.*, 1904 (15), 617, hence only special references will be cited in the following discussion.

three factors : First, it is extremely difficult to obtain melanin in a pure condition ; second, the process of purification requires the action of strong acids and alkalies, which undoubtedly modify the composition of the melanin ; thirdly, melanin is probably not a single substance of definite composition, but includes several related but different bodies. The values found vary for carbon from 48.95 to 60.02 per cent.; for hydrogen from 3.05 to 7.57 per cent.; for nitrogen, 8.1 to 13.77 per cent. Hofmeister gives, as a characteristic of melanins, that their elementary molecular composition is always nearly in the proportions $N : H : C = 1 : 5 : 5$.

A particularly prominent constituent of melanin is sulphur, which has been found in as high proportions as 10 per cent. in melanin from sarcomas, and even 12 per cent. in sepia from the squid ; in melanin from hair the sulphur is usually about 2–4 per cent.; but in choroid melanin, and in some other forms, sulphur seems to be absent. The proportions of sulphur obtained from the same specimen purified by different methods show wide variations, and hence v. Fürth considers that neither the sulphur nor the iron are indispensable constituents of the melanin. Probably the melanin molecule contains atom-complexes that have a tendency to bind certain sulphur and iron compounds (*e. g.*, cystin or hematin derivatives).

There is much reason to believe that the melanin is derived from certain groups of the proteid molecule that seem readily to form colored compounds. The aromatic compounds of the proteid molecule, such as tyrosin, phenylalanin, and tryptophan, readily condense with elimination of water and absorption of oxygen, to produce dark-colored substances. When proteids are heated in strong hydrochloric acid, we obtain a dark-brown material, which closely resembles the melanins both in elementary composition and in general properties, so that it is referred to as “artificial melanin” or “melanoid substance.” These substances, like the natural melanins, when decomposed by fusing with caustic potash, yield skatol, indol, and pyrrol derivatives, which are undoubtedly derived from the tyrosin and tryptophan of the proteid molecule. Therefore, it seems probable that both the melanoid substances and the true melanins are formed from the chromogen groups of the proteid molecule through processes of condensation, elimination of water, and the taking up of oxygen.

Tyrosinase.—In the sepia sacs of the cuttle-fish, in mealworms which form a melanin-like pigment, and in plants that produce the black Japanese lacquer, have been found *oxidizing*

enzymes that have the property of producing black pigment by their action upon tyrosin and other aromatic compounds. These enzymes may, therefore, possibly be responsible for the production of melanin in animal tissues, by causing oxidative changes in the chromogen groups of the proteid molecule that are liberated by autolysis (see "Tyrosinase" p. 79). v. Fürth urges strongly the view that both normal and pathological melanin formation depends upon the action of tyrosinase or allied enzymes in conjunction with autolytic enzymes; the latter split free the chromogen groups of the proteid molecule, which are then oxidized by the tyrosinase, undergo condensation, and take up sulphur- and iron-holding groups and also other organic compounds, the entire complex forming the melanin.

Properties of Melanin.—When isolated in a pure condition, melanin is a dark-brown substance of amorphous structure, no matter how black the material from which it is derived may be.¹ It is quite insoluble in all ordinary reagents except alkalis, in which some melanins dissolve easily, and some with difficulty. Strong boiling hydrochloric acid scarcely affects melanin. By the action of sunlight or oxidizing agents on melanin-containing sections the pigment can be bleached out. The chief decomposition-products formed on fusing with alkalis are indol, skatol, and "melanic acid"; no cystin, leucin, tyrosin, or other amino-acids can be isolated. Most authors, therefore, consider the melanins as heterocyclic compounds standing in some relation to the indol nucleus.

If melanin is injected subcutaneously into animals (rabbits and guinea-pigs), there appears in the urine a substance which turns dark brown after the urine has stood for some time (Kobert, Helman). The pigment is apparently reduced, particularly by the liver, to a colorless melanogen, which is eliminated in the urine. The same process occurs when melanin is produced in excess and enters the blood, as in the case of melanosarcoma, a colorless melanogen being formed which is excreted in the urine, constituting "melanuria." Occasionally the urine is dark when first passed, because of the presence of melanin, but usually it must be subjected to oxidizing agencies (bromine water, nitric acid, hypochlorites, etc.), or exposed to air to bring out the brown color. Helman² says that true melanogen may be considered to be present in urine: (1) If the careful

¹Spiegler (Hofmeister's Beitr., 1903 (4), 40) claims to have isolated a white chromogen, closely related to melanin chemically, which causes the white color of wool and hair.

²Cent. f. inn. Med., 1902 (23), 1017; Arch. internat. Pharmacodynam., 1903 (12), 271.

addition of ferric chloride causes the development of a black precipitate. (2) If this precipitate dissolves in sodium carbonate, forming a black solution. (3) If from this solution mineral acids precipitate a black or brownish-black powder. All three reactions must be obtained, for substances other than melanin may give the first two.

The coloring power of melanin is very great, for urine containing but 0.1 per cent. of melanin has the color of dark beer (Hensen and Nölke), and the entire skin of a negro contains only about 1 gram of melanin (Abel and Davis¹). Excessive quantities of melanin may be in part deposited in the lymph-glands and skin, causing diffuse pigmentation; it may also be deposited in the endothelium lining the blood-vessels. Kobert injected melanin into albino rabbits, but did not succeed in getting any deposition in the choroid or skin. Helman found some evidence of toxicity when large doses of melanin dissolved in sodium carbonate are injected into animals, but this is possibly due to the alkali rather than to the melanin.

Melanotic Tumors.—Tumor melanin does not differ from melanin produced by normal cells in any essential respect. Usually it contains much sulphur, even as much as 10 per cent., yet Helman in eight specimens found but four that contained both sulphur and iron, in three only sulphur, in one only iron and no sulphur; therefore, tumor melanins show the same variations in composition as do normal melanins. Iron is frequently found microscopically in the pigment in melanosarcoma, but this is chiefly due to admixture of blood-pigment coming from extravasations of blood. The peculiar fact that melanosarcoma is very common in white or gray horses, but very seldom occurs in dark-coated horses, has not been explained. The frequent occurrence of melanuria and melanemia in patients with melanosarcoma is not due to any peculiar property of sarcoma melanin, but to the enormous quantity of melanin that is produced by the tumor and set free in the degenerating portions. Thus, while Abel and Davis¹ estimate that there is only about 1 gram of melanin in the entire skin of a negro, Nencki and Berdez have obtained from a sarcomatous liver 300 grams of melanin, and estimate that the entire body contained 500 grams. Helman² states that the melanin may constitute 7.3 per cent. by weight of the fresh substance of some melanosarcomas. According to Lubarsch and to Helman, melanotic tumors rarely contain glycogen.

¹ Jour. Exp. Med., 1896 (1), 361.

² Arch. internat. Pharmacodynam., 1903 (12), 271.

Addison's disease is associated with the deposition of a pigment in the skin that is generally considered to be a melanin, differing from that produced normally in the skin only in quantity and not in origin or composition.¹ No satisfactory explanation of the relation of the adrenal to this pigmentation seems yet to have been made, although it is natural to assume that when the function of the adrenal is destroyed, substances accumulate in the blood that have a stimulating effect on the pigment-forming cells. Abnormal proteid katabolism, with excessive accumulation of the chromogenic constituents of the proteid molecule, has been suggested, as also have alterations in the influence of the sympathetic nervous system upon the chromophore cells, for nerve lesions (*e. g.*, neurofibroma) are often accompanied by pathological pigmentation of the skin.² As exact chemical studies of the pigment in Addison's disease have not been made, however, we have no positive proof that it is a melanin, hence any speculation as to the cause of its formation is premature. Carbone³ claims to have isolated from the urine in Addison's disease a pigment that contains much sulphur, and which he considers similar to or identical with the melanogen of melanuria. v. Kahlden,⁴ however, has observed crystals resembling hematin in the pigmented tissues.

Ochronosis is a condition characterized by a black pigmentation of the cartilages, first described by Virchow in 1866. In 1904 Osler⁵ reported two cases, and found but seven others in the literature to that time. The origin and nature of this pigment remain still undecided. Virchow suspected that the condition was due to a permeation of cartilage by hematin derivatives, but Hansemann, finding a case associated with melanuria, considered that the pigment is probably of metabolic origin. Hecker and Wolf studied the urine of a similar case, and concluded that the pigment must be melanin. Albrecht,⁶ however, suggested a relation of ochronosis to *alkaptonuria*, having found hemogentisic acid in the urine of a case reported by him (see "*Alkaptonuria*"). Osler's two patients were brothers with *alkaptonuria*, the evidence of ochronosis consisting of discoloration of the cartilages of the ears. Langstein⁷

¹ Concerning histogenesis of the pigment see Pförringer, *Cent. f. Path.*, 1900 (11), 1.

² See résumé by Schmidt, *Ergeb. der Pathol.*, 1896 (Bd. 3, Abt. 1), 551.

³ *Giorno R. Acad. med. di Torino*, 1896.

⁴ *Virchow's Arch.*, 1888 (114), 65.

⁵ *Lancet*, 1904 (i), 10 (literature).

⁶ *Zeit. f. Heilk., Path. Abt.*, 1902 (23), 366.

⁷ *Hofmeister's Beitr.*, 1903 (4), 145.

has examined a specimen of urine preserved from Hansemann's case, and found no evidence of alkaptonuria.¹

Pick² has recently added another case to the literature, and he summarizes the results of his study of this case and of the literature, as follows: Ochronosis is a definite form of melanotic pigmentation, the pigment of ochronosis being in most of the cases very closely related to melanin. The pigment, or its chromogen, circulating freely in the blood, is imbibed not only by cartilage, but also by loose connective tissue, voluntary and involuntary muscle-cells, and epithelial cells, without any decrease in vitality of these cells being observable; however, degenerated tissues show the greatest amount of pigmentation. The diffuse pigment can become granular after a time; it is iron-free, but under certain circumstances may contain fat. *This melanin arises from the aromatic nucleus of the proteid molecule (tyrosin, phenylalanin), and the related hydroxylized products, under the influence of tyrosinase.* In two cases the constant absorption of minute quantities of phenol from surgical dressings seems to have been the cause of the condition. Besides this formation of pigment from such "exogenous" aromatic substances, however, it is probable that in alkaptonuria the "endogenous" aromatic substances (alkaptonuric acids) present may be converted into pigment by the tyrosinase. In many of the cases of ochronosis the pigment or a precursor may be excreted in the urine, which then undergoes spontaneous darkening when exposed to the air. The kidneys may also become pigmented, and granular masses of pigment may be present in the renal tubules.

Malarial pigmentation has been studied, particularly by Ewing,³ who states that in malarial fever one may meet with granular, sometimes crystalline, pigment particles, free in the vessels or englobed in various cells, not giving the Prussian-blue reaction, nor dissolving in chloroform, ether, or carbon bisulphide, but dissolving in ammonium sulphide. This pigment may have any one of the following origins:

- (1) Pigment elaborated by the intracellular parasite.
- (2) Hematoidin derived from the remnants of infected red cells.
- (3) Hematoidin or altered hemoglobin deposited in granular or crystalline form from red cells dissolved in the plasma.
- (4) Bilirubin or urobilin granules or crystals.

Of these, the pigment formed by the parasites has been considered by many as a true melanin, but this cannot be considered

¹ Also see Langstein, Berl. klin. Woch., 1906 (43), 597.

² Berl. klin. Wochenschr., 1906 (43), 478.

³ Jour. Exp. Med., 1902 (6), 119.

as established, especially as Ewing finds it to have the same relation to solvents as do the blood-pigments.

LIPOCHROME

In normal plant and animal tissues occur pigments that are either fats or compounds of fat. In animals they occur normally in the corpus luteum; in the epithelium of the seminal vesicles, testicles, and epididymis; in ganglion-cells, especially in the sympathetic nervous tissue; and in fat tissue. Pathologically, such pigments are found particularly in the muscle-cells in brown atrophy of the heart, and less abundantly in the epithelium of atrophied livers and kidneys (Lubarsch¹ and Sehrt²). All are characterized by staining by such fat stains as sudan III and scarlet R, and usually, but not constantly, by osmic acid; they are dissolved by the usual fat solvents. It is questionable if all pigments that stain for fat should be considered as true lipochromes, however, for their other reactions are variable. Typical plant lipochromes, including the pigments of *Staphylococcus pyogenes aureus* and *citreus*, are colored blue by concentrated sulphuric acid with formation of small blue crystals of *lipocyantin*. With iodine-potassium-iodide solution they are colored green. Lipochrome of frog-fat stains blue with the iodine-potassium-iodide solution (Neumann³); lipochrome of the corpus luteum (called *lutein*) occasionally gives a faint blue with sulphuric acid or Lugol's solution (Sehrt); but the fat-holding pigments of the other tissues mentioned above do not give either of these reactions. Possibly these last are not true lipochromes, therefore, but rather pigments chemically or physically combined with fat. Cotte⁴ believes that the true lipochromes of plants and animals have a cholesterin base, but the presence of glycerin in plant and bacterial lipochromes can be demonstrated by the acrolein test—possibly, therefore, both cholesterin and neutral fats are present. Melanins and pigments derived from hemoglobin do not stain with sudan III and are not soluble in ether, etc., and hence can be readily distinguished from the fatty pigments.

The pigment that causes the peculiar green color characteristic of certain malignant growths, *chloroma*,⁵ was considered by Chiari, Huber, and others as a fatty substance related to or identical

¹Cent. f. Pathol., 1902 (13), 881.

²Virchow's Arch., 1904 (177), 248.

³Virchow's Arch., 1902 (170), 363.

⁴Compt. Rend. Soc. Biol., 1903 (55), 812.

⁵Literature by Dock, Amer. Jour. Med. Sci., 1893 (106), 152; and Dock and Warthin, Med. News, 1904 (85), 971.

with the lipochromes. It commonly fades on exposure to air, and also when in the usual preservative fluids, to which it does not impart its color. It contains no iron, is soluble in absolute alcohol and in ether, and is usually, but not always (v. Recklinghausen), stained black with osmic acid. The pigment of *xanthelasma multiplex* also seems to be a fatty substance (Poensgen¹).

Chromophile cells may be considered in this connection. Kohn² has described certain cells with a decided affinity for chromic acid and its salts, found abundantly in the sympathetic nervous system, in the carotid gland, and in the medulla of the adrenal. They are also present in tumors derived from these organs. Extracts from such organs have a marked effect in raising blood pressure, and, according to Wiesel,³ they are greatly involved in Addison's disease. The nature of the chromophile substance is unknown, but it can only be fixed by chromic acid or chromates; cells hardened by other means show merely spaces in the places occupied by this substance. Mulon⁴ believes it to be the same as the adrenalin.

BLOOD PIGMENTS⁵

Red corpuscles behave much as do other non-nucleated fragments of cells, undergoing disintegration rapidly and constantly when under normal conditions, as well as when subjected to various harmful influences (see "Hemolysis"), or when outside of the vessels in extravasations of blood. The processes and products of their disintegration are, therefore, much the same whether occurring under normal or pathological conditions. The hemoglobin molecule is large and complex, and from it are derived many substances of the nature of pigments; indeed, hemoglobin itself may appear free as a pigment.

Hemoglobin is a compound proteid, consisting of a proteid group (*globin*) and a coloring-matter (*hematin* or *hemochromogen*). The proteid globin is of a basic nature, and seems allied to the histons. The hematin is, therefore, presumably acid, and the compound proteid, hemoglobin, is strikingly like the nucleoproteids in nature. Hemoglobin ordinarily does not crystallize readily, especially the hemoglobin of man, and it is doubtful if it ever does so in the living tissues, although possibly this may occur in the center of large hematomas. In bodies that have undergone postmortem decomposition, and occasionally in

¹ Virchow's Arch., 1883 (91), 354.

² Prag. med. Woch., 1902 (27), 325.

³ Zeit. f. Heilk., Path. Abt., 1903 (24), 257.

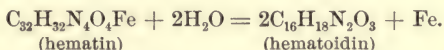
⁴ Compt. Rend. Soc. Biol., 1904 (56), 113.

⁵ Literature by Schmidt, Ergebnisse der Pathol., 1894 (I₂), 101; and 1896 (III₁), 542; Schulz, Ergebnisse der Physiol., 1902 (I₁), 505.

specimens kept for microscopic purposes, irregular orange-yellow crystalline masses of hemoglobin may be found. This occurs particularly if the blood has been acted upon by hemolytic agents or has undergone putrefactive changes, and then is hardened in alcohol. The crystals are either oxyhemoglobin, or more often an isomeric or polymeric modification, *parahemoglobin* (Nencki). Hemoglobin also enters cells unchanged, imparting a diffuse yellowish color.

In the decomposition of hemoglobin the first step is the splitting of the globin (which does not form pigments) from the hematin, from which many pigments may be derived.

Hematin.—The formula given for this substance by Nencki, $C_{32}H_{32}N_4FeO_4$, has been generally accepted, although it is not certain that the hematin of all animals is the same. It is found frequently as an amorphous, dark-brown or bluish-black substance, in large, old extravasations of blood, but seldom in small hemorrhages. As a pathological pigment, however, hematin is by no means so frequently found as its derivatives. Wherever formed its duration is transient, for it gradually splits up into an iron-free pigment (*hematoidin*) and an iron-containing pigment (*hemosiderin*). This change may be represented by the following equation, according to Nencki and Sieber¹:



Hematoidin may be found in old, large extravasations, as orange-colored or red rhombic plates, first described by Virchow. Sometimes, however, hematoidin occurs in the form of yellowish granular masses. It seems to be nearly or quite identical with the bile-pigment, bilirubin, and it is probably the source of this substance under normal conditions. When formed in excessive amounts, either through increased destruction of corpuscles in the vessels or in extravasations, the amount of bile-pigment is increased (see "Icterus"). Possibly some of the hematoidin becomes transformed directly into *urobilin*, and is then eliminated in the urine.

Hemosiderin² is relatively insoluble, and, therefore, is more slowly removed when formed in hemorrhages, and more abundantly deposited in the tissues when formed after excessive hemolysis. According to Neumann, hemosiderin is produced only under the influence of living cells and in the presence of

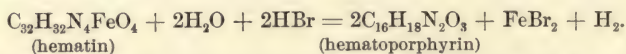
¹ Arch. exp. Path. u. Pharm., 1888 (24), 440.

² See Neumann, Virchow's Arch., 1888 (111), 25; 1900 (161), 422; 1904 (177) 401; also Arnold, *ibid.*, 1900 (161), 284.

oxygen, while hematoidin arises independent of cellular activity.¹ Milner² considers that, under similar conditions, an iron-containing pigment is also formed, which differs from hemosiderin in having the iron so combined that it cannot react with the usual reagents; this pigment may later change into hemosiderin. Up to the present time we do not know the chemical nature of hemosiderin, nor its exact fate in the body, but it is probably utilized in the manufacture of new hemoglobin, for it is known that the iron liberated when hematin is broken up in the body under experimental conditions is deposited and not eliminated (Morishima³).

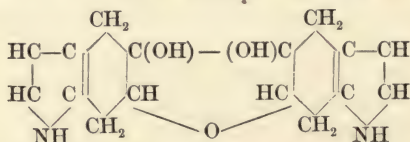
Unstained hemosiderin generally appears in the form of brown or yellowish-brown granules, and not as crystals. After a time it is taken up and deposited to a large extent in the liver, spleen, bone-marrow, and kidney, either as hemosiderin or possibly as some other iron compound of similar nature. From these sites it seems to be later taken up to be utilized in the manufacture of new red corpuscles.

Hematoporphyrin.—This substance is readily formed from hematin through removal of the iron, as shown by the following reaction :



The formation of hematoporphyrin from hematin also occurs readily in the animal body, provided that the hematin is in a reduced condition, according to Laidlaw,⁴ but not when oxidized.

The structural formula is believed to be as shown below :



Physiologically, this pigment is of great interest, because of the close chemical relation that it has been found to bear to *chlorophyll*,⁵ with which hemoglobin is so closely related functionally. It is also interesting to consider that whereas carnivora obtain much hemoglobin in their food, herbivora obtain

¹ The accumulation of iron in the liver which follows poisoning with hemolytic agents, is not prevented or diminished by preliminary removal of the spleen (Meinertz, Zeit. exp. Path. u. Ther., 1906 (2), 602).

² Virchow's Arch., 1903 (174), 475.

³ Arch. exp. Path. u. Pharm., 1898 (41), 291.

⁴ Jour. of Physiol., 1904 (31), 464.

⁵ For literature see Abderhalden, "Lehrbuch der physiol. Chemie," 1906.

much chlorophyll. Pathologically, hematoporphyrin is of interest as a urinary pigment, being found normally in the urine in traces, but present in considerable quantities in many diseases,¹ such as rheumatism, tuberculosis, various liver diseases, and, most strikingly, after the administration of sulphonal or trional. When in abundance it may color the urine a rich Burgundy red.

Pseudomelanosis.—When loosely bound iron is present in the tissues, and in the same tissues sulphides are produced through bacterial action, a discoloration with sulphide of iron will result, which is called *pseudomelanosis*, because the pigment resembles true melanin in its blackness. This is most frequently observed as a postmortem phenomenon in and about the abdominal cavity, and in the ordinary postmortem discoloration both the liberation of the iron from its firm organic combination, and the production of hydrogen sulphide, are the work of bacteria. Pseudomelanosis may also occur *intra vitam*, particularly in the margins of infected areas, and it may also be observed in the liver and spleen, and about the peritoneum, in bodies examined immediately after death, before any evident postmortem decomposition has set in. This seems to depend upon the previous *intra vitam* formation of hemosiderin, which is then combined by sulphur liberated from tissue proteids through bacterial action.² If hydrogen sulphide acts upon hemoglobin that has not been decomposed, a greenish compound of *sulphur-methemoglobin* is formed (Harnack³), which is the cause of the greenish color seen in the abdominal walls and along the vessels of cadavers. This union of hemoglobin and hydrogen sulphide occurs only when oxygen is present (oxyhemoglobin). The sulphur-hemoglobin compound is readily decomposed by weak acids, even by CO₂, with the formation of *methemoglobin*, which in turn readily becomes decomposed to form hematin.

Hemofuscin is the name given by von Recklinghausen to the brownish pigment found in involuntary muscle-fibers, particularly in the wall of the intestine. It does not react for iron, and is insoluble in alcohol, ether, chloroform, or acids; therefore it is not a lipochrome. von Recklinghausen, and also Goebel,⁴ ascribe this pigment to an alteration of hemoglobin which enters the cells in dissolved form, but Rosenfeld,⁵ who has submitted the material to analysis after isolation, found

¹ See Garrod, Jour. of Physiol., 1892 (13), 598.

² Ernst, Virchow's Arch., 1898 (152), 418. Literature.

³ Zeit. physiol. Chem., 1899 (26), 558.

⁴ Virchow's Arch., 1894 (136), 482.

⁵ Arch. exp. Path. u. Pharm., 1900 (45), 46.

3.70 per cent. of sulphur, from which he considers that it is related to the melanins or melanoid substances. The substance is readily dissolved by alkalis, and contains no iron. According to Taranoukhine,¹ the pigment in the myocardium in *brown atrophy of the heart* is also derived from proteids, and is neither a lipochrome nor a hemoglobin derivative. Other observers, however, consider this pigment a lipochrome (*q. v.*).

Hemochromatosis.²—This name was given by von Recklinghausen to a condition in which the organs and tissues throughout the body are abundantly infiltrated with two pigments: one, iron-containing, identical with hemosiderin; the other seems to be the same as the hemofuscin described above. The hemosiderin is found chiefly in the parenchyma cells of the glandular organs, especially the liver and pancreas, which organs usually show marked interstitial proliferation. The hemofuscin is found in the smooth muscle-fibers of the gastrointestinal tract, blood-vessels, and genito-urinary tract. Under the heading of local hemochromatosis, von Recklinghausen grouped such conditions as brown atrophy of the heart, and pigmentation of the intestinal wall, which probably are quite distinct from the generalized hemochromatosis, since the local form occurs as a physiological process in old age. In a considerable proportion of the cases of generalized hemochromatosis there occurs diabetes, called by Hanot, "bronzed diabetes," because of the coloration of the skin.³ It has been suggested that the pigmentation is due to decomposition of the blood-corpuscles in the diabetic blood, but recent writers seem agreed that the pigmentation and sclerotic changes precede the diabetes, which is secondary to the atrophic and sclerotic changes in the pancreas. There can be little question that both the pigment formation and the tissue changes depend upon some intoxication, the origin and nature of the toxic agent being entirely unknown. In many cases it has seemed probable that alcohol might have been the inciting cause.

Opie's conclusions concerning this subject are as follows: (1) There is a distinct morbid entity, hemochromatosis, characterized by wide-spread deposition of an iron-containing pigment in certain cells, and an associated formation of iron-free pigments in a variety of localities in which pigment is found in moderate

¹ Roussky Arch. Patol., 1900 (10) 441.

² Literature given by Opie, Jour. Exp. Med., 1899 (4), 279; and Beattie, Jour. Pathol. and Bact., 1903 (9), 117.

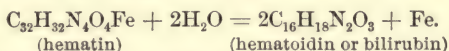
³ Literature by Opie and Beattie (*loc. cit.*); also by Anschütz, Deut. Arch. klin. Med., 1899 (62), 411; Hess and Zurhelle, Zeit. klin. Med., 1905 (57), 362.

amount under physiological conditions. (2) With the pigment accumulation there occur degeneration and death of the containing cells and consequent interstitial inflammation, notably of the liver and pancreas, which become the seat of inflammatory changes accompanied by hypertrophy of the organ. (3) When chronic interstitial pancreatitis has reached a certain grade of intensity, diabetes ensues, and is the terminal event in the disease.

ICTERUS¹

Pigmentation of the tissues of the body in jaundice depends upon the presence in them of bile-pigments, which have been formed in the liver and reabsorbed either into the lymph or blood (or both). Although a pigment that seems to be chemically identical with bilirubin (*hematoidin*) may be formed from hemoglobin liberated on the breaking up of red corpuscles, yet this is probably never formed in sufficient amounts outside of the liver to give rise to general icterus. However, the local greenish-yellow pigmentation occurring in the vicinity of extravasations of blood, due to hematoidin formation, may be looked upon as a "local jaundice."

Bile-pigments.—*Bilirubin* is of a reddish-yellow color, and it is the chief pigment of human bile. Its formula is $C_{16}H_{18}N_2O_3$, and its relation to hematin, from which it is formed, is shown by the following formula, which, according to Nencki and Sieber, expresses the manner in which blood pigment is converted into bilirubin by the liver under normal conditions, and into hematoidin (its isomer) in the tissues and fluids of the body in pathological conditions:



Bilirubin is not soluble in water, but dissolves in the alkaline body fluids as a soluble compound, "bilirubin alkali." It is very slightly soluble in ether, benzene, carbon disulphide, amyl-alcohol, fatty oils, and glycerin, but is more soluble in alcohol and in chloroform.

Biliverdin, $C_{16}H_{18}N_2O_4$, as its formula indicates, is an oxidation product of bilirubin. Bilirubin in alkaline solutions will oxidize into biliverdin merely on exposure to the air, and the change from yellow to green of icteric specimens when placed in oxidizing solutions (*e. g.*, dichromate hardening fluids) is due to the formation of the green biliverdin. Biliverdin is the chief pigment of the bile of carnivora, but it is also present in varying amounts in human bile.

The various other biliary pigments, namely, *bilifuscin*, *biliprasin*, *choleprasin*,² *bilihumin*, and *bilicyanin*, are probably not normal constituents of bile, but are oxidation products of bilirubin, and are found

¹ Literature by Stadelmann, "Der Icterus," Stuttgart, 1891; Minkowski, *Ergebnisse der Pathol.*, 1895 (2), 679.

² See Küster, *Zeit. physiol. Chem.*, 1906 (47), 294.

chiefly in gall-stones (*q. v.*). A pigment similar to urobilin may be present in normal bile. The total amount of pigments present in bile is probably not far from one gram per liter; rather under than above this amount.

Etiology of Icterus.—Although hematin, which is isomeric if not identical with bilirubin, may be formed outside of the liver when red corpuscles are broken up in hemorrhagic extravasations, and possibly also when they are broken up within the vessels by hemolytic agents, yet it is generally considered that *a true general icterus does not occur without the liver being implicated*. This view rests on evidence of various sorts. First, the classical experiments of Minkowski and Naunyn,¹ which demonstrated that in geese the production of hemolysis by means of arseniuretted hydrogen leads to icterus, but if the livers of the geese have been previously removed, no icterus follows the poisoning. Second, the repeated demonstration that in icterus produced by septic conditions, poisoning, etc., which was formerly looked upon as a “hematogenous” icterus, the urine contains bile salts as well as pigment, indicating an absorption of bile from the liver. Third, the finding of histological evidence that in so-called hematogenous icterus there occur occlusions or lesions of some sort in the bile capillaries, which can account for the reabsorption of the bile into the general circulation.² Joannovics³ gives, as a result of a comparative study of icterus from bile obstruction and icterus from hemolysis, the following chief differences: Icterus due to hemolysis appears sooner than icterus from bile-duct occlusion, and reaches a much higher degree; the obstruction in hemolytic icterus is intra-acinous; in stasis it is chiefly inter-acinous; in hemolytic icterus there is a large splenic tumor due to accumulation of degenerated red cells in the spleen, where they become disintegrated preliminary to the formation of bile-pigment. If the spleen is removed, hemolytic agents do not cause icterus, because the corpuscles are not then prepared for pigment formation.

Therefore, it is believed that the pigments that produce the general discoloration of icterus are, at least for the most part, manufactured by the liver, whatever the cause of the reabsorption of the bile from the liver into the blood may be. That

¹ Arch. f. exp. Pathol. u. Pharm., 1886 (21), 1.

² See Eppinger, Ziegler's Beitr., 1903 (33), 123; Gerhardt, Münch. med. Woch., 1905 (52), 889. Lang (Zeit. exp. Path. u. Ther., July, 1906, Bd. 3) has demonstrated the presence of fibrinogen in the bile in phosphorus-poisoning, which perhaps accounts for the “bile thrombi” observed by Eppinger in toxic icterus.

³ Zeit. f. Heilk., Path. Abt., 1904 (25), 25.

hemolytic agents cause icterus is explained by the fact that on account of the large amounts of free hemoglobin brought to the liver, excessive amounts of bile-pigment are formed, which render the bile so viscid that it blocks up the fine bile capillaries; on account of the low pressure at which bile is secreted, a slight obstruction of this kind is sufficient to stop entirely the outflow of bile, which then enters the lymphatics of the liver and also the blood-stream itself.¹ It is also possible that the hemolytic poisons injure the liver-cells so much that the minute intra- and intercellular bile capillaries become disorganized, and permit of escape of bile into the lymph-spaces and its absorption into the blood-vessels. Swelling of the degenerated liver-cells may also be an important factor in the occlusion of the bile capillaries, and swelling of the living cells of the bile capillaries may also coëxist.

Toxicity of Bile.—In any event, we must appreciate that in icterus not only are abnormally large quantities of bile-pigment present in the blood, but also the other less conspicuous constituents of the bile. The relative toxicity of the bile-pigments and the bile salts is not as yet uniformly agreed upon.

Bile-pigments.—Bouchard² and others have claimed that the bile-pigments are far more toxic than the bile salts, which is contradicted by Rywosch and others. As Rywosch found that doses of 0.6 gram of bile-pigments per kilo had almost no effect on rabbits, it is doubtful if the amount absorbed by a patient with icterus can have serious effects, since it is estimated that the normal daily excretion of bile-pigment in man averages but about 0.5 gram. The amount of pigment in the blood in icterus is correspondingly minute.³

Bile salts are undoubtedly toxic, generally producing depression of the central nervous system, with resulting coma and paralysis; they are also decidedly toxic to cells of all sorts, causing hemolysis and marked destruction of tissue-cells. Small quantities of bile salts stimulate the central end of the vagus, and larger amounts influence the heart itself; hence in icterus we observe a slowing, and often an irregularity, of the pulse, and the blood pressure is lowered. Although there has been

¹ See Mendel and Underhill, *Amer. Jour. Physiol.*, 1905 (14), 252.

² Literature and discussion by Stadelmann, *Zeit. f. Biol.*, 1896 (34), 57.

³ A series of analyses by Gilbert and others (*Compt. Rend. Soc. Biol.*, 1905 (38), July 7, *et seq.*) gave the following results: Normal blood-serum contains 0.027–0.08 gram bilirubin per liter, which is the source of the normally produced urobilin; in obstructive icterus they found 0.068 gram of bilirubin per liter, or about 0.2 gram in the blood of the entire body; in biliary cirrhosis 0.33 gram per liter, in icterus neonatorum 0.2 to 0.5 gram, in pneumonia 0.068 gram was found.

much dispute as to whether the chief effects of icterus upon the heart depend upon action of the bile salts upon the vagus, or upon the intracardiac ganglia, or upon the muscle itself,¹ yet Weintraud demonstrated that in some cases of icterus administration of atropin, which paralyzes the vagus, stops the bradycardia, indicating the importance of the effects of the bile salts upon the vagus in causing this feature of cholemia. According to Meltzer and Salant,² bile also contains a tetanic element, which disappears from stagnating bile; the bile salts contain this tetanizing agent in less amount than does the whole bile.

Since the bile salts cause hemolysis, and since in even "hematogenous" jaundice they enter the blood, it can readily be seen that in this way an increased formation of bile-pigment may be incited which leads to further obstruction to the outflow of bile from the liver, and a "vicious circle" may thus be established. The necroses observed in the liver in icterus, "*icteric necrosis*," are generally ascribed to the cytotoxic effects of the bile salts, although it is difficult always to eliminate infection extending along the bile-ducts to the liver tissue. The itching and irritation of the skin in icterus may be due to the effect of the bile-pigments deposited in it.

A remarkable tendency to spontaneous hemorrhages, frequently observed in icterus, probably depends upon injury to the capillary endothelium by the bile salts; while the protracted, often uncontrollable, hemorrhage that may occur from operation wounds in icteric patients, is related to the slowed coagulation of the blood observed in icterus. The cytotoxic effect of the bile salts is also shown by the albuminuria of icteric persons, which frequently results from the renal lesions the bile produces.

Croftan³ summarizes the physiological effects of bile acids as follows: (1) A powerful cytolytic action, affecting both blood-corpuscles and tissue-cells. (2) A distinct cholagogue action. (3) In small doses (1-500) they aid coagulation. (4) In large doses (1-250 and over) they retard coagulation. (5) Slow the heart action. (6) In small doses they act as vasodilators; in large doses, as vasoconstrictors. (7) Reduce motor and sensory irritability. (8) Act on the higher cerebral centers, causing coma, stupor, and death.

¹ See Minkowski, *Ergeb. der Pathol.*, 1895 (2), 709.

² *Jour. Exp. Med.*, 1906 (8), 128; review and literature concerning toxicity of bile.

³ *New York Med. Jour.*, 1906 (83), 810; see also Faust, "Die tierische Gifte," Braunschweig, 1906, p. 29.

It is difficult to decide how much of the profound intoxication that is sometimes present in icterus ("cholemia" and "icterus gravis") to ascribe to the reabsorbed bile, for frequently there is an accompanying infection, and even if there is no infection the impairment of liver function by the obstruction to bile outflow must also be reckoned with. The liver is not only the great destroyer of toxic substances absorbed from the alimentary canal, but it is also an important seat of nitrogenous metabolism, interference with which may lead to accumulation of many toxic nitrogenous substances in the blood.¹ The long duration of severe icterus in some cases of occlusion of the bile-ducts, with relatively slight evidences of intoxication, would seem to indicate, however, that on the whole the bile is not so much responsible for the intoxication observed in icterus as are the associated conditions. On the other hand, in not a few instances it has been observed that escape of large quantities of bile into the peritoneal cavity may be followed by symptoms similar to those of icterus gravis; in these cases only the bile can be held responsible for the intoxication.²

The Pigmentation in Icterus.—Living tissues have but a slight tendency to take up bile-pigments, much of the tissue-staining observed at autopsy being due to postmortem imbibition from the blood and lymph. Quinke³ found that after subcutaneous injection of bilirubin only the connective tissue, both cells and intercellular fibrils, becomes diffusely colored; later, it fades out of the cells, leaving only the fibrils stained. Muscle-cells, fat-cells, and vessel-walls take up the pigment only after their death. If the jaundice continues for a long time, the subcutaneous deposits of bilirubin may undergo a slow oxidation, the color changing to an olive or to a dirty grayish green. The pigment in the connective tissues is at first in solution, but may be deposited in a granular form after a considerable amount has accumulated.

The question whether in icterus the skin may be colored by other pigments than bilirubin, especially by its reduction product, *hydrobilirubin* or urobilin, seems to have been decided negatively. This substance is formed from bilirubin by bacterial reduction in the intestines, is absorbed, and is probably the source of the urobilin in the urine. No matter how much hydrobilirubin is produced in the intestine, however, or how

¹ See Bickel, *Exper. Untersuch. über der Pathol. der Cholaemie*, Wiesbaden, 1900.

² See Ehrhardt, *Arch. klin. Chir.*, 1901 (64), 314.

³ Virchow's *Arch.*, 1884 (95), 125.

much urobilin is present in the urine, the tissues do not become pigmented by them. Bile-pigment is probably not absorbed as such from the intestine in sufficient quantity to cause icterus. Such bile-pigment as enters the blood from the liver is excreted through the kidneys chiefly, but also in the sweat. Ordinarily, other secretions (milk, tears, saliva, sputum) are not colored in jaundice, but if the secretions are mixed with inflammatory exudations, they may then be colored (*e. g.*, pneumonic sputum). When the bile-pigment is resorbed from the skin, it is at least in part transformed into urobilin, which appears in the urine in increased amounts during the period of recovery from jaundice. Part of the bile-pigment is probably eliminated by the liver after the cause of obstruction has been removed from the bile-passages.

Digestive Disturbances in Obstructive Icterus.—In case the icterus depends upon the occlusion of the main bile-passages by stones, tumors, etc., the situation is complicated by the effects of the absence of this natural secretion in the intestinal canal. Carbohydrate and proteid digestion seem to be but little affected, especially the former, but the proportion of the ingested fat that appears in the feces increases from the normal 7–11 per cent. to 60–80 per cent. The products of bacterial decomposition of the undigested fat may lead to injury of the intestinal wall and disturbance of its function. Failure of absorption of fat also favors intestinal putrefaction by enveloping the proteid substances so that they are not readily digested and absorbed. The relation of bile to intestinal putrefaction is still not exactly determined. Frequently, but by no means always, there is an increased intestinal putrefaction which may result in diarrhea and the appearance of excessive quantities of indican and phenol in the urine. The idea once held that the bile salts acted as intestinal antiseptics has not been established by experimental investigations; however, it is possible that through their function as natural cathartics, by stimulation of peristalsis, they prevent stagnation and putrefaction of proteids.

CHAPTER XVII

THE CHEMISTRY OF TUMORS

CHEMICAL investigations of tumors have been relatively few in number, but, so far as they have yet been made, there has been little detected that indicates any important deviation of the chemical processes of tumors from those of normal cells of similar origin. Likewise, the chemical composition of tumor tissue resembles closely, on the whole, the composition of related normal tissues. It is hardly to be imagined that the course of chemical changes is greatly different in tumor cells from that in normal cells, in view of the abundant evidence that the metabolic products of tumor cells are identical with those of the cells from which they arose. Thus, metastatic growths of thyroid tissue will produce thyroiodin in any part of the body, liver carcinoma metastases produce bile, tumors from the choroid or from pigmented moles produce melanin, etc. The capacity of tumor cells to produce complicated products of metabolic action specific for the parent cells from which they arose, as illustrated above, indicates beyond question that the course of their chemical activities is very much like that of normal cells. So, too, the composition of the cells is found to be similar indeed to that of the parent cells, both in regard to primary and secondary constituents. Thus, Bang found that sarcomas derived from lymph-glands contain the particular nucleoproteids that are found normally only in lymph-glands; hypernephromas contain, like adrenal tissue, much fat, lecithin, and cholesterin; squamous cell carcinomas develop great amounts of kerato-hyalin; carcinomas of mucous membranes may contain much mucin, etc.

Many have sought in cancer tissues a poison that might account for the cachexia characteristic of new-growths. Extracts have been obtained that were destructive to red corpuscles (hemolytic), and that were sometimes slightly toxic to animals, but the results have not seemed sufficiently striking to account for the appearance of cachexia. Because of the interference with circulation, brought about in tumors by pressure of the growing tissues upon their blood-vessels, areas of necrosis frequently develop, and these, undergoing autolysis, yield substances

that are hemolytic and toxic.¹ Whether these are the cause of cancer cachexia, however, may be questioned; but they are sufficient to account for most of the experimental results as yet obtained. No substance has yet been isolated from or detected in malignant growths that is peculiar to them and not found in normal cells, and still less has any substance been detected that accounts in any way either for the occurrence of tumors or for the effects that they produce.

Nevertheless, numerous observations have been made concerning the chemistry of tumors, which, although they do not as yet throw any important light on the fundamental problems of tumor pathology, are of much interest. These may be briefly summarized as follows:

A. CHEMISTRY OF TUMORS IN GENERAL

(1) **Proteids.**—Earlier studies showed that tumor growths contain the same sorts of proteids as do normal tissues, and apparently in about the same proportions. Recently investigations have been made concerning the more minute chemical features. Wolff² has studied the proteids obtained in the juice expressed from cancer-cells by the Buchner press. In normal tissues such cell-juice contains, almost constantly, nearly equal proportions of albumin and globulin. In carcinoma, however, the albumin is usually three or more times as abundant as is the globulin. Of the globulins, it is particularly the euglobulin that is reduced, in some instances being nearly absent. In tumor-free liver tissue between carcinomatous growths the proportion of albumin was found increased above that normal for liver tissue. Wolff found no qualitative differences between cancer proteids and normal cell proteids. Joachim has found, similarly, that in cancerous ascites the proportion of albumin is much higher than in ascites from other causes. This is rather remarkable, in view of the fact that in cachexia the proportion of albumin in the blood and in exudates sinks much more rapidly than does the proportion of globulin.³

In all probability the nucleoproteids of tumors share the specific characteristics of the nucleoproteids of the tissues from which they arise—at least this is the case with the nucleoproteids of lymphosarcoma, according to Bang.⁴ The characteristic con-

¹ Rülff (Zeit. f. Krebsforschung, 1906 (4), 417) considers the proteolytic enzymes of much importance in the causation of cancer cachexia.

² Zeitschrift f. Krebsforschung, 1905 (3), 95; Medizinische Klinik, 1905 (1), 13.

³ Umber, Zeit. klin. Med., 1903 (48), 364.

⁴ Hofmeister's Beitr., 1903 (4), 368.

stituent of lymph-glands, spleen, and thymus is a compound of nucleic acid and histon (*histon nucleinate*). If to a watery extract of an organ a few drops of CaCl_2 solution are added, the formation of a precipitate indicates the presence of a lymphatic tissue. If this precipitate is soluble in 1 per cent. NaCl , it is a nucleinate corresponding in type to that of the lymph-glands and spleen; if not soluble, it is of the type of the thymus or leucocytes. Extracts from no other organs give a precipitate with calcium chloride. Spindle-cell sarcomas were found not to give this reaction, but round-cell sarcomas of lymphatic origin do, for they contain the specific nucleinate abundantly. Bang believes that this reaction can be used to distinguish sarcoma arising from lymphoid tissue. This seems to have been confirmed by Beebe,¹ who found nucleo-histon only in lymph-gland tissue, but the distinction between thymus and lymph-gland nucleo-histon is probably not so easily made as Bang intimates.

Because of their richly cellular structure, cancers may contain more nucleoproteid than the tissues from which they arise. Thus Petry² found 50 per cent. of nucleoproteid in carcinoma of the mammary gland, as against 30 per cent. in normal tissue.

Bergell and Dörpinghaus³ have studied the nature of the proteids in tumors by determining the proportion of the various amino-acids that compose them. Because of the amount of material necessary for the ester method, they were obliged to use a mixture of various primary and secondary cancers and one sarcoma. The proteid of this tumor-mixture was characterized by the very high proportion of alanin, glutaminic acid, phenylalanin, and asparaginic acid, there being from 5 to 10 per cent. of each. Leucin was very low, 5–10 per cent., as against 20 per cent., or higher, found in most normal tissues. Glycocoll and tyrosin were present in small quantities, and serin was probably also present. Neuberg⁴ found in cancer proteid 1.3 per cent. of tyrosin, 17 per cent. of leucin, scarcely 1 per cent. of glutaminic acid, and 4.92 per cent. of glycocoll. Further investigations along these lines are greatly to be desired.

On account of the amount of autolysis going on in tumors the products of proteid splitting are usually present. Beebe⁵

¹ Amer. Jour. Physiol., 1905 (13), 341.

² Zeit. physiol. Chem., 1899 (27), 398.

³ Deut. med. Woch., 1905 (31), 1426.

⁴ Arb. a. d. Path. Inst. zu Berlin, 1906, p. 593.

⁵ Amer. Jour. Physiol., 1904 (11), 139.

found in a number of tumors leucin, tyrosin, tryptophan, proteoses (biuret reaction), and in one glycocoll. Because of the deficient circulation in the tumors, the amino-acids accumulate in the cancer tissues in sufficient amounts to be detected, and may be found even when no macroscopic evidences of degeneration are present. Possibly on account of this poor absorption no proteoses, peptones, or amino-acids could be found in the urine of cancer patients by Wolff;¹ but Ury and Lilienthal² found a positive reaction for albumose in the urine in about two-thirds of all carcinoma cases examined by them; however, it may be absent even in advanced stages. Petry³ states that in normal mammary glands all the nitrogen is in a coagulable form, whereas in sarcoma but 13 per cent. is coagulable. This non-coagulable nitrogen is partly in the form of proteoses and peptones, partly as substances not giving the biuret reaction.

(2) **Other Organic Constituents.**—These, in general, resemble the organic constituents of the tissue from which the tumor arises, for a structural resemblance to the parent tissue always exists, and as structural features depend largely on the proportion of the chemical components, a structural similarity fairly implies a chemical similarity. For example, adrenal tissue contains much fatty material, especially lecithin and cholesterolin, and hypernephromas show a similar composition; Gatti⁴ noted that the proportion of lecithin in a hypernephroma is similar to that in the adrenal; the fat of a lipoma is, in its qualitative features, almost identical with the normal fat of the same individual; tumor melanin shows no characteristic chemical distinction from normal melanin, etc.

Glycogen has been particularly studied in tumors, especially because of the erroneous idea advanced by Brault that the quantity of glycogen is in direct proportion to the malignancy. From a summary of all the evidence, it seems that two chief factors determine the presence and amount of glycogen in tumors. One is the embryonic origin of the tumors; thus tumors of cartilage, striated muscle, or of squamous epithelium, which tissues normally contain much glycogen, are likewise provided with an abundance of this material. Second, the occurrence of areas of impaired cell-nutrition favors the accumulation of glycogen in the degenerating tumor-cells, just as it leads to a similar accumulation in all other tissues (Gierke⁵). The most extensive consideration of this topic is reported by Lubarsch,⁶

¹ Zeit. f. Krebsforschung, 1905 (3), 95.

⁴ Virch. Arch., 1897 (150), 417.

² Arch. f. Verdauungskr., 1905 (11), 72.

⁵ Ziegler's Beitr., 1905 (37), 502.

³ Loc. cit.

⁶ Virchow's Arch., 1906 (183), 188.

who found glycogen microscopically in 447 (or 29 per cent.) of 1544 tumors examined. It was present in but 3 out of 184 fibromas, osteomas, gliomas, hemangiomas, lipomas, and lymphangiomas, and in but 2 out of 260 adenomas from various parts of the body. It occurred in all teratomas, rhabdomyomas, hypernephromas, and syncytiomas. In 138 sarcomas glycogen was present in 70 (50.7 per cent.); of 415 carcinomas it was found in 181 (43.6 per cent.). In the squamous epithelial cancers 70 per cent. contained glycogen, while the mucoid or colloid cancers were always free from glycogen. The glycogen undoubtedly enters the cells from without, probably entering as sugar, and being converted into glycogen by intracellular enzymes. We have no reliable studies of the actual quantity of glycogen in various tumors, although Meillère¹ states that the microscopic and chemical examination of tumors give corresponding comparative results, which Gierke states is generally true with glycogen estimations.

Pentoses.—Neuberg² reports finding, as a product of autolysis of a carcinoma of the liver, a pentose which was not produced by autolysis of either normal liver tissue or the primary growth in the stomach. Beebe³ found that in carcinoma of the mammary gland the percentage of pentose (*xylose*) is somewhat higher than the amount in normal mammary glands (about 0.23 per cent.). Carcinoma in the liver did not show any constant excess of pentose above that of normal liver tissue (about 0.38 per cent.). A primary carcinoma of the liver showed quite the same pentose and phosphorus content as normal liver tissue. In general, no constant relation of pentose to origin, malignancy, or degeneration of tumors was observed. The most significant suggestion of this and other work by the same author is that the composition of a metastatic growth may be modified by its environment, so that it may differ from the primary growth more than from the normal tissue in which it has taken root.

(3) **Inorganic Constituents.**—These have been studied under exceptionally favorable conditions, in that the age of the tumor could be accurately estimated, in the inoculable carcinoma of mice (Jensen), by Clowes and Frisbie.⁴ They found that rapidly growing tumors contain a high percentage of potassium and little or no calcium, whereas in old, slowly growing,

¹ Compt. Rend. Soc. Biol., 1900 (52), 324.

² Berl. klin. Woch., 1904 (41), 1081; 1905 (42), 118.

³ Amer. Jour. Physiol., 1905 (14), 231.

⁴ Amer. Jour. Physiol., 1905 (14), 173.

relatively necrobiotic tumors the relation is reversed, the potassium decreasing greatly while the calcium increases. Magnesium is present only in traces, while the proportion of sodium fluctuates much less, but is usually greater than either the potassium or calcium, although in very old tumors the latter may become excessive. The most rapid growth, however, seems to occur in tumors in which both calcium and potassium are present in the ratio of $\frac{K}{Ca} = \frac{2}{1}$ or $\frac{3}{2}$.

Beebe¹ analyzed a number of human tumors with the following results: Phosphorus was found in proportion to the amount of nuclear material, varying from 0.139 per cent. (uterine fibroid) to 1.06 per cent. (sarcoma). Iron varied from 0.013 per cent. to 0.064 per cent., probably depending on the amount of blood and nucleoproteids. Calcium is most abundant in old degenerated tumors, and potassium in rapidly growing tumors. These results, supported by Clowes and Frisbie's findings, indicate the importance of potassium for cell growth.

Schwalbe² found that cancer-cells contain iron in a condition demonstrable by the Berlin-blue reaction, and occurring independent of hemorrhages. Tracy³ found that tumors reacted microscopically for iron, either free or in the form of an albuminate, only in areas where hemorrhage had occurred. Nuclear or organic iron could be detected in the nuclei, occurring in a network arrangement. In other words, iron occurs in tumors, both quantitatively and qualitatively, exactly as in normal cells of the same type. The same writer⁴ found in tumors, by microchemical reactions, that phosphorus in the form of nucleoproteids likewise shows no essential differences from its distribution in normal tissues.

In this connection may be mentioned the observations of Hemmeter,⁵ who found that the cells of carcinoma of the mammary gland will shrink when placed in physiological salt solution or in the serum of the patient, whereas normal cells swell when placed in cancer-juice. This suggests that the osmotic pressure, and, by inference, the amount of inorganic constituents, is lower than in normal tissues.

(4) **Enzymes.**—The rapid and extensive autolysis that occurs in tumors, as shown both morphologically and by the presence of the products of proteid splitting in them, indicates

¹ Amer. Jour. Physiol., 1904 (12), 167.

² Cent. f. Path., 1901 (12), 874.

³ Jour. Med. Research, 1905 (14), 1.

⁴ Martha Tracy, Jour. Med. Research, 1906 (14), 447.

⁵ Amer. Jour. Med. Sci., 1903 (125), 680.

that tumor cells resemble all other cells in possessing intracellular proteolytic enzymes. We have no evidence that these enzymes are different, either qualitatively or quantitatively, from those of corresponding normal tissues. They are discussed more fully under the subject of autolysis (Chap. iii). The influence of radium rays in hastening autolysis of cancers is even greater than that of *x*-rays (Neuberg¹).

Other enzymes are also present in tumor cells. Buxton² examined a large number of tumors for their enzymes by the plate (*auxanographic*) method, and found considerable variations in different growths. All contained amylase (splitting starch) and lipase (splitting butyrin). Most, but not all, tumors coagulated milk and liquefied casein, and also liquefied gelatin (rennin, proteases). Peroxidase was nearly always, and catalase always, present. Digestion of fibrin, coagulated serum, and coagulated egg-albumen could not be observed. Practically all tumors split glycogen. Tyrosinase could not be demonstrated. The fact that early embryonic tissues were found poor in enzymes³ speaks against the common assumption that tumors represent strictly an embryonic formation.

MacFadyen and Harden⁴ studied the juices obtained by grinding up tumor cells made brittle by liquid air, and found by direct methods (chiefly in breast cancers) invertase, maltase, amylase, proteases acting in both acid and alkaline solutions, catalase, oxidase, with perhaps traces of lipase and peroxidase, but no lactase.

Tumors arising from the gastric mucosa, according to Waring,⁵ contain both pepsin and rennin; those from the pancreas, both primary and secondary growths, contain trypsin, steapsin, amylase, and rennin.

(5) **Internal Secretion.**—If tumors are derived from an organ with an important internal secretion, the tumor cells in many cases produce the same internal secretion, which seems to have the same functional properties as the normally produced secretion. Thus a metastatic growth from a thyroid tumor has been known to functionate in place of the resected gland; Gierke⁶ found in about 20 grams of material from metastatic thyroid tissue in the vertebral column about 5 mg. of iodine, which was a trifle larger proportion than was present in the

¹ Arb. a. d. Path. Inst. zu Berlin, 1906, p. 593.

² Jour. Med. Research, 1903 (9), 356.

³ *Ibid.*, 1905 (13), 543.

⁴ Lancet, 1903 (ii), 224.

⁵ Jour. Anat. and Physiol., 1894 (28), 142.

⁶ Hofmeister's Beitr., 1902 (3), 286.

thyroid itself. Adrenal cancers do not usually cause Addison's disease, because they functionate in place of the destroyed gland (Lubarsch). But in the peculiar and characteristic production of cachexia, often apparently out of all proportion to the amount of tumor tissue, there would seem to be evidence that a peculiar and abnormal product of metabolism is formed by cancer-cells. As yet, however, it has been impossible to demonstrate any characteristic toxic substance in cancers.¹

Because of the constant disintegration of the tumor tissues, products of autolysis are formed, and undoubtedly enter the circulation in small quantities; possibly they are a factor in the systemic manifestations of malignant growths, but it is highly doubtful whether they are sufficient, either in toxicity or quantity, to account for all the systemic effects.

Since all normal tissue-cells produce substances through their metabolism that enter the circulation, it is quite certain that tumor-cells do likewise, and it is highly probable that the presence of abnormal quantities of such products, even if they are of quite normal composition, may cause disturbances in the body. As yet, however, no such substances, either normal or abnormal, have been isolated, nor has their presence been demonstrated. Numerous isolated observations of ptomaines or similar substances in the urine of cancer patients may be found in the literature,² but their importance is extremely questionable.

Hemolytic Substances.—A number of observers have described the finding of hemolytic substances in cancer extracts. Bard³ observed that in hemorrhagic carcinomatous exudates in serous cavities the blood is rapidly hemolyzed, which is not the case in exudates from other causes. Kullmann⁴ found that extracts of carcinomas contain hemolytic substances acting energetically both in the body and *in vitro*; these are soluble in alcohol and in water, are not complex in composition, are not specific for human corpuscles, but are toxic for all varieties of corpuscles. Micheli and Donati⁵ likewise found hemolytic substances in 8 of 15 tumors, of which 5 acted on all varieties of corpuscles, and 3 acted on only certain varieties; they regard the hemolytic substances as the products of autolysis in the tumors. It is well known that among the products of autolysis of normal tissues are hemolytic substances. Whether the severe

¹ See Blumenthal, Festschr. f. Salkowski, Berlin, 1904.

² See Kullmann, Zeit. klin. Med., 1904 (53), 293.

³ La Semaine Méd., 1901 (21), 201.

⁴ Loc. cit.

⁵ Riforma Med., 1903 (19), 1037.

anemia frequently present in carcinoma is due, either largely or in part, to these products of autolysis is unknown, but it is very probable that they have some effect.

(6) **Metabolism in Cancer.**—Speaking against any specific nature in the cause of cancer cachexia are numerous observations, indicating that the cachexia is in no way different from the cachexia of other conditions. The behavior of the nitrogen metabolism seems to be quite the same as in tuberculosis and other wasting diseases. There is the same excessive elimination of aromatic substances (phenol, indican) and oxyacids (Lewin,¹ Blumenthal²), which Lewin considers to arise from the abnormal metabolism of proteids, and not from putrefactive decomposition in the tumor or in the intestines. There is also the same excessive elimination of mineral salts observed in pulmonary tuberculosis, and termed “demineralization” by Robin.³

Israel, and also Engelmann, have reported the occurrence of a marked increase in the lowering of the freezing-point of the blood in carcinoma (as low as -0.60° to -0.63° , the normal being -0.56°), which they attributed to the presence of excessive products of proteid decomposition in the blood. Engel,⁴ however, found no such increased lowering of the freezing-point in his cases, and questions the significance of the results of Israel and Engelmann.

(7) **Immunity against Cancer.**—Numerous attempts to produce specific anti-bodies for malignant cells have been made by injecting into animals ground-up tumor tissue, or the blood of cancer patients, or normal tissues of the same origin as the cancer.⁵ The results have generally been negative, and the most favorable reports have been entirely unconvincing. Many difficulties, as yet but incompletely surmounted (see Chap. ix), lie in the way of securing specific antiserum for particular cells; the difficulties in the case of malignant growths is even greater, and at the time of writing the possibilities of therapeutic success by this method are not promising.

Kullmann⁶ found that the serum of animals immunized against cancer tissue exhibits strong hemolytic properties. This

¹ Deut. med. Woch., 1905 (31), 218.

² Festschr. f. Salkowski, Berlin, 1904.

³ Quoted by Lewin, *loc. cit.* Clowes *et al.* (5th Ann. Rep., N. Y. State Dept. of Health, 1903-4) report observing a slight chloride retention in cancer patients, and review the literature of metabolism in cancer.

⁴ Berl. klin. Woch., 1904 (41), 828.

⁵ Literature by Engel, Deut. med. Woch., 1903 (29), 897.

⁶ *Loc. cit.*

formation of hemolysins in immunization against tissues, even when comparatively (or completely) free from blood, has frequently been observed when normal cells have been injected, and it is not due to a biological modification of non-specific hemolytic substances present in the cancer, as Kullmann suggests.

B. CHEMISTRY OF CERTAIN SPECIFIC TUMORS

In the literature are to be found a few studies of chemical features of some forms of tumors, which may be briefly discussed to advantage.

(1) BENIGN TUMORS

(a) **Fibromas.**—The few specimens studied show but a small amount of nucleoproteid, as might be expected from the small amount of their nuclear material. Because of the tendency of fibromas to undergo retrogressive changes, the amount of calcium is likely to be large. No studies as to the special features of their collagen, as compared with normal connective-tissue collagen, seem to have been made. Lubarsch¹ found no glycogen (microscopically) in any of 66 fibromas he examined.

A uterine fibroid analyzed by Beebe² contained 14.56 per cent. of nitrogen, 0.981 per cent. of sulphur, 0.139 per cent. of phosphorus, 0.013 per cent. of iron, 0.12 per cent. of calcium oxide, 0.44 per cent. of potassium, and 1.115 per cent. of sodium. The proportions of nitrogen and sulphur are high as compared with most tumors; the phosphorus, iron, and potassium are low, corresponding to the small amount of nucleoproteid and the slow rate of growth. If degeneration is marked, the amount of calcium is greatly increased. Krawkow³ found a trace of chondroitin-sulphuric acid in a uterine fibroid. Lubarsch found glycogen occasionally in richly cellular uterine leiomyomas, and in the vicinity of degenerating areas; however, 76 out of 85 showed no glycogen. Pfannenstiel⁴ analyzed the alkaline fluid of a cystic fibromyoma, which coagulated spontaneously; it contained sugar, but no mucin or pseudomucin. The cysts were dilated lymph-spaces, and the fluid corresponded to lymph in composition. A similar result was obtained by Oerum,⁵ who found in the fluid serum-albumin, serum-globulin, and 0.358 per cent. of fibrin; the total proteids

¹ Virchow's Arch., 1906 (183), 188.

² Amer. Jour. Physiol., 1904 (12), 167.

³ Arch. exp. Path. u. Pharm., 1898 (40), 195.

⁴ Arch. f. Gyn., 1890 (38), 468.

⁵ Maly's Jahresber., 1884 (14), 462.

constituted 6.3056 per cent. Sollmann¹ found in the "colloid" of a cystic degenerated fibromyoma both pseudomucin and paramucin (see "Ovarian Cysts"), which differed somewhat from the same substances found in ovarian tumors.

(b) **Chondromas**, like normal cartilage, always contain much glycogen (Lubarsch). Mörner² found chondroitin-sulphuric acid in several chondromas that he examined, although Schmiedeberg had failed to do so.

(c) **Lipomas** have been studied by Schulz³ and by Jaeckle.⁴ The former found in a retroperitoneal lipoma 75.75 per cent. of fat, 2.25 per cent. of connective tissue, and 22 per cent. of water. Of the fat, 7.31 per cent. was in the form of the free fatty acids and 92.7 per cent. as neutral fats. The fatty acids of the fat consisted of 65.57 per cent. oleic acid; 29.84 per cent. stearic acid; 4.59 per cent. palmitic acid. Cholesterin was only qualitatively demonstrable. In the connective tissue was found chondroitin-sulphuric acid. Lubarsch found glycogen in lipomas only when they were degenerated. Jaeckle observed the formation of calcium soaps in a calcifying lipoma, the calcium being distributed as follows: calcium soaps, 29.5 per cent.; calcium carbonate, 28.61 per cent.; calcium phosphate, 41.89 per cent. The fats of lipomas he found practically identical with those of the subcutaneous tissues, except sometimes for a deficiency in lecithin, as shown by the following figures:

COMPOSITION OF FATS IN—

	Subcutaneous tissue.	Lipoma I.	Lipoma II.	Lipoma III.
Refraction, at 40°	50.6	50.1	50.9	50.5
Saponification number . .	197.3	197.7	197.7	195.9
Reichert-Meisser number .	0.25	0.33	0.35	0.35
Iodin number	63.7	59.0	64.0	64.1
Olein	74.1	68.6	74.4	74.5
Oleic acid	70.9	65.7	71.2	71.3
Acid number	0.39	0.31	0.48	0.67
Free acid	0.196	0.155	0.24	0.34
Palmitic acid	18.5	24.9	..	18.5
Stearic acid	6.2	5.1	..	5.9
Lecithin	0.084	..	..	0.015
Cholesterin	0.32	..	0.34	..

Edsall found the composition of the fat in the fatty tumors of *adiposis dolorosa* but little different from that of normal fat.⁵

¹ Amer. Gynecol., 1903 (2), 232.

² Zeit. physiol. Chem., 1895 (20), 357.

³ Pflüger's Arch., 1893 (55), 231.

⁴ Zeit. physiol. Chem., 1902 (36), 53.

⁵ Quoted by Dercum and McCarthy, Amer. Jour. Med. Sci., 1902 (124), 994.

(d) **Ovarian cyst contents** have been studied more than almost any other tumor products, because in their gelatinous or slimy substance are contained numerous interesting forms of proteids, many of which are combined with carbohydrates and related to the true mucins. These substances are frequently referred to under the names of *pseudomucin*, *paralbumin*, *metalbumin*, and *ovarian "colloid,"* and belong to the class of "*mucoids*."¹ In view of the fact that the fluids in the Graafian follicles of the ovary do not contain these particular forms of proteid, their presence in cysts derived from adventitious structures (Pflüger's epithelial tubes) suggests a specific form of metabolism on the part of the epithelium of these structures.

Serous cysts, formed by dilatation of Graafian follicles, usually are small in size, and the contents resemble those of the normal follicles (Oerum²), consisting of a serous fluid with a specific gravity usually from 1.005 to 1.014 (occasionally 1.020 or more), and containing 1.0–4.0 per cent. of solids. Occasionally in these cysts the contents become solidified through absorption of the water, and a gelatinous or glue-like "*colloid*" content results. Mucoids are never present (Pfannenstiel³).

Proliferating cystomas contain the peculiar characteristic mucoid proteids mentioned above. Usually the contents are fluid, but of a peculiar slimy, stringy character, due to the mucoid substance, and often opalescent or slightly turbid. The specific gravity is generally high—1.015–1.030. The reaction is usually slightly alkaline to litmus, and neutral or slightly acid to phenolphthalein. If hemorrhage has occurred into them, the fluid is discolored, and may contain blood-pigments in crystalline and amorphous forms. Small cysts often show a condensation of the proteids into a semisolid "*colloid*" material, but sometimes their contents resemble those of a serous cyst. Often masses of proteids fall out of solution, forming yellowish flocculi or large deposits half filling the cysts. As with all stagnant fluids of this type, cholesterin crystals are frequently found. The characteristic proteids are members of the class of pseudomucins, which are constantly present (Oerum).

Chemistry of the Mucoids of Ovarian Cysts.—Pseudomucin has the following elementary composition: C, 49.75; H, 6.98; N, 10.28; S, 1.25; O, 31.74 per cent. (Hammarsten). In common with the true mucins it yields a sugar-like reducing body, which has been investigated

¹ Concerning mucoids see Mann's "Chemistry of the Proteids," 1906, pp. 541–551.

² See Maly's Jahresbericht, 1884 (14), 459.

³ Arch. f. Gynæk., 1890 (38), 407 (literature).

by numerous chemists (Müller, Panzer, Zangerle, Leathes, Neuberg, and Heymann¹). Panzer considers that this reducing substance is in the form of a sulphuric-acid compound, similar to, but not identical with, chondroitin-sulphuric acid. Hammarsten, however, did not find this substance constantly present. Leathes determined for the carbohydrate group the composition $C_{12}H_{23}NO_{10}$, named it "*paramucosin*," and considers it a reduced *chondrosin* (which is the carbohydrate group of chondroitin-sulphuric acid). Neuberg and Heymann established, however, that the reducing body must come from *chitosamin* ($C_6H_{13}NO_5$), and do not consider paramucosin a constant constituent of ovarian mucoids. The amount of reducing substance varies greatly in the mucoids found in different cysts; in some the mucoid yields but about 3 to 5 per cent., in others as much as 30 or 35 per cent., of reducing substance.

Pseudomucin dissolves readily in weak alkalis, and differs from true mucin in that it is not precipitated by acetic acid, and from the simple proteids in that its solutions are not coagulated by boiling. With water a slimy, stringy semi-solution is formed, resembling in appearance the material found in ovarian cysts. Leathes distinguishes two forms of ovarian mucoids: One, *paramucin*, occurs as a firm, jelly-like substance, which is converted by peptic digestion into the easily soluble pseudomucin. Ovarian "*colloid*" probably consists of a thickened pseudomucin, often mixed with other proteids. Pfannenstiel² considers the "*colloid*" material as representing a modified pseudomucin, strongly alkaline and relatively insoluble, which he calls "*pseudo-mucin β*." He also describes a very soluble mucoid found only in certain ovarian cysts, naming it "*pseudo-mucin γ*."

The reasons why these variations in the pseudomucins exist is not understood; they cannot be explained as due to variations in the cell type in the cyst wall, although pseudomucin is probably the result of true secretion. The smallest cavities of ovarian cystadenomas contain nearly pure pseudomucin, which presents a clear, glassy structure; the larger the cysts become, and the more turbid and thinner the fluid is, the more simple are the proteids it contains. True mucin is never present in ovarian cysts. Pseudomucin occurs only in the glandular proliferating cystomas and the papillary proliferating cystadenomas, in the former appearing constantly and abundantly, in the latter not constantly and never abundantly (Pfannenstiel). *Paralbumin* (Scherer) is a mixture of pseudomucin with variable amounts of simple proteids. *Metalbumin* (Scherer) is the same body that is called pseudomucin by Hammarsten. *Paramucin* (Mitjukoff)³ is a mucoid differing from mucin and pseudomucin in reducing Fehling's solution directly, without having the carbohydrate group first split off by boiling with an acid.

¹ Hofmeister's Beitr., 1902 (2), 201 (literature).

² Loc. cit.

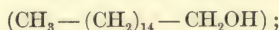
³ Arch. f. Gynæk., 1895 (49), 278.

Substances similar to pseudomucin have been occasionally found in cancerous ascitic fluid and in cystic fibromyomas (Sollmann); and they are abundant as constituents of the contents of the peritoneum in the condition known as "*pseudomyxoma peritonei*,"¹ when the material is in reality the product of cells implanted on the peritoneal surface through the bursting of an ovarian cyst (or a cyst of the vermiform appendix (Fränkel²)). The physically similar substance found in pathological synovial membranes by Hammarsten differs in yielding no reducing substance. Parovarian cysts arising from the Wolffian body present an entirely different content, which is a clear, watery fluid, with specific gravity usually under 1.010; the solids amount to but 1 or 2 per cent., and consist chiefly of salts (the ash being often over 80 per cent.), mostly sulphates and chlorides. They are usually (or always) free from pseudomucin, mucin, or other sugar-containing substances, and other proteids occur only in small amounts, unless the cyst is inflamed. Apparently mucoids do not form in cysts lined by ciliated epithelium (Pfannenstiel).

Intraligamentary papillary cysts contain a yellow, yellowish-green, or brownish-green liquid, which contains little or no pseudomucin; the specific gravity is usually high (1.032–1.036) and the fluid contains 9 to 10 per cent. of solids. The principal constituents are the simple proteids of blood-serum (Hammarsten).

According to the same author, the rare *tubo-ovarian cysts* contain a watery serous fluid with no pseudomucin.

(e) **Dermoid cysts** of the ovary contain, as their chief and most characteristic constituent, a yellow fat, which melts at 34°–39° and solidifies at 20°–25°. Ludwig and Zeynek³ have examined over sixty such tumors, and found that the fatty material constantly contains two chief constituents: one, crystallizing out readily, seems to be *cetyl alcohol*,



the other, remaining as an oily fluid, seems to be closely related to cholesterin, although not consisting of one substance alone. Small quantities of *arachidic acid* ($\text{C}_{20}\text{H}_{40}\text{O}_2$), as well as *stearic*, *palmitic*, and *myristic acid* ($\text{C}_{14}\text{H}_{28}\text{O}_2$), existing as glycerides, are also present. These substances are secreted

¹ Literature by Peters, Monatschr. f. Geb. u. Gyn., 1899 (10), 749; Weber, St. Petersb. med. Woch., 1901 (26), 331.

² Münch. med. Woch., 1901 (48), 965.

³ Zeit. physiol. Chem., 1897 (23), 40.

by the glands of the cutaneous structures of the cyst, and resemble in composition sebaceous material, which is characterized by containing a large proportion of cholesterin partly combined with fatty acids.

(f) "**Butter**" Cysts.—In the mammary gland retention cysts form, filled with products of alteration of the milk, including butyric acid and lactose (Klotz¹), and these are called "butter cysts" or milk cysts. Analysis of the contents of such a cyst by Smita² gave the following results, as compared with human milk:

	Cyst contents.	Human milk.
Fat	72.97	3.90
Casein	4.37	0.63
Albumin	1.91	1.31
Milk-sugar	0.88	6.04
Ash	0.36	0.49
Water	20.81	87.09
Fats consisted of—		
Stearin and palmitin	Cyst. 37.0	Cows' milk. 50.0
Olein	53.0	42.2
Butyrin	9.0	7.8

Occurring independent of lactation usually, but not always, are the "*soap cysts*," which contain chiefly calcium and magnesium soaps, but also neutral fats, free fatty acids, and traces of cholesterin (Freund³).

(2) MALIGNANT TUMORS

The chief general features of the composition of these growths have been considered in the discussion of the chemistry of tumors in general (pages 412–420). A malignant tumor differs from a similar benign tumor chiefly in having usually a larger proportion of the primary cell constituents, and a smaller proportion of the secondary constituents and intercellular substances, since these are largely the product of the functional activity of the cells, which, in malignant tumors, do not often develop sufficiently to functionate extensively. Hence malignant tumors usually show a rather high proportion of the characteristic constituents of nucleoproteids; *i. e.*, phosphorus and iron. If rapidly growing, they contain much potassium; if undergoing much retrogression, little potassium and a larger amount of calcium (Beebe, Clowes and Frisbie). On account of the extensive disintegration, the products of autolysis are usually much more abundant than in

¹ Arch. klin. Chir., 1880 (25), 49.

² Wien. klin. Woch., 1890 (3), 551.

³ Virchow's Arch., 1899 (156), 151.

benign tumors. The composition varies greatly with the origin, although to a less extent than with the benign tumors. As Bang and Beebe have shown, the tumors arising from lymphatic tissues show the chemical characteristics of these structures, and contain histon nucleinate. Tumors from squamous epithelium develop keratin in direct proportion to the amount of maturity the cells reach. Even the most complex and specific products of metabolic activity may be developed by malignant tumors (*e. g.*, thyroiodin, adrenalin, bile), and in a form and condition capable of performing function. As Buxton has shown, malignant tumors produce a great variety of intracellular enzymes. The idea that glycogen is present in tumors in proportion to their malignancy has been disproved by Lubarsch, Gierke, and others; among the malignant tumors glycogen is found particularly in chorioepitheliomas, hypernephromas, and squamous cell carcinomas. Of particular importance is the observation of Beebe, that the composition of metastatic growths is modified by the organ in which they are growing, so that they tend to resemble the organ serving as their host.

As to the special varieties of malignant growths, there is little as yet determined concerning their chemistry beyond what has been stated above. Their variations in composition are largely the direct result either of their resemblance to some normal tissue or of degenerative changes that they have undergone.

"Colloid" carcinoma may be mentioned specially, in view of the confusion caused by the lax use of the term "colloid" (*q. v.*, p. 354). The fluid contents of colloid cancers of the gastro-intestinal tract are usually chiefly epithelial mucus, containing mucin mixed with a greater or less quantity of proteids from degenerated cells and serous effusion. This mucin is acid in reaction, is precipitated by acetic acid, and has an affinity for basic dyes. The colloid cancers of the mammary gland, in which the "colloid degeneration" involves the stroma, probably contain a connective-tissue mucin, analogous to that of the umbilical cord, as also do the myxosarcomas, if we may judge by their origin and staining reactions, but no exact chemical study of these substances can be found. Colloid cancers of the ovary, arising usually from the same structures as the ovarian cysts, contain pseudomucin or allied bodies (see "Ovarian Cysts"). Colloid tumors of thyroid tissue contain the typical colloid of normal thyroid tissue, even when metastatic in other organs; in the tumor colloid is a relatively normal proportion of iodine (Gierke¹).

¹ Hofmeister's Beitr., 1902 (3), 286.

Hypernephromas possess several interesting chemical features. For example, at a time when the origin of these tumors was in dispute, Gatti¹ brought forward the fact that such a tumor analyzed by him contained 3.4735 per cent. of lecithin, which agreed very well with the amount of lecithin in normal adrenals. Beebe² found in the watery extract of a hypernephroma the following substances: tryptophan, proteoses, glycogen, leucin, and tyrosin, indicating the occurrence of autolysis. About 29 per cent. of fat was present, which was all extractable without pepsin digestion, and the fat contained about 18 per cent. of its weight as cholesterin. Lecithin was also present, but not quantitatively determined. Croftan³ states that hypernephroma tissue resembles adrenal tissue in causing glycosuria when extracts are injected subcutaneously into rabbits, in splitting starch into sugar, and in decolorizing a blue iodine-starch solution. The last of these reactions is given by so many other tissues, however, that its differential value is doubtful.

Melanotic tumors produce melanin, which seems not to differ at all from the melanin found in normal pigmented structures (see Chap. xvi). Helman⁴ found as high as 7.3 per cent. by weight of melanin in melanosarcomas.

MULTIPLE MYELOMAS AND MYELOPATHIC "ALBUMOSURIA"

Multiple myelomas are of particular chemical interest, because of the appearance in the urine of such cases of the peculiar proteid first described as an *albumose* by Bence-Jones,⁵ and now, because of lack of grounds for its definite classification, generally known as the "*Bence-Jones body*" or "*Bence-Jones proteid*." This variety of tumor differs from the standard types of malignant tumors in that it involves the marrow of many bones simultaneously, in a very diffuse manner, without usually giving evidence of a true metastasis. In many respects it resembles the leukemias, pseudoleukemia, and chloroma, and it is extremely uncertain as to where in the classification of tumors and of the diseases of the blood-forming organs this disease should be placed. Histologically, the tumors show evidence of being derived from the specific cells of the marrow, either from the

¹ Virchow's Arch., 1897 (150), 417.

² Amer. Jour. Physiol., 1904 (11), 139.

³ Virchow's Arch., 1902 (169), 332.

⁴ Arch. internat. Pharmacodyn., 1903 (12), 271.

⁵ References not generally cited, as there exist several complete résumés of the literature; see Simon, Amer. Jour. Med. Sci., 1902 (123), 939; Weber *et al.*, *ibid.*, 1903 (126), 644; Moflatt, Lancet, 1905 (i), 207.

plasma cells (Wright) or from the neutrophile myelocytes or their predecessors (Muir).

Properties of the "Bence-Jones Proteid."—Not to go into details, which are given in the literature cited, the important facts concerning the "*Bence-Jones proteid*," and its appearance in the urine ("*myelopathic albumosuria*," Bradshaw), are as follows :

It is a proteid, the exact nature of which has not been determined ; at first considered an albumose because of its peculiar reactions to heat, its nature has since been contested, but the weight of evidence seems to be in favor of the contention of Simon that it is most closely related to the water-soluble globulin of the blood. Its most characteristic properties are the following :

The coagulation temperature is low, varying from 49°–60° in various cases, and being considerably modified by the amount of salts and urea present in the solution.

In many cases the coagulum is redissolved on heating, and reappears on cooling, but this characteristic feature is not always present, and often disappears in cases where at first it is present.

A precipitate is formed by strong (25 per cent.) nitric acid, which disappears on heating and reappears on cooling. Strong hydrochloric acid causes a dense precipitate, which is quite typical (Bradshaw).

No precipitate is produced by acetic acid, even in excess, and the addition of acetic acid to a hot coagulated specimen causes prompt solution of the coagulum.

Unlike albumoses, this substance does not dialyze ; the salt-free solution left in the dialyzing bag does not precipitate.

A purplish-violet color is usually given with the biuret reaction, but it may be more reddish in color, especially if little copper is present.

Sulphur is readily split off by alkalies, reacting with lead acetate to produce lead sulphide (Boston).

After standing in alcohol, by which the body is precipitated, it loses its solubility (differing in this respect from albumose).

As to the exact nature of the body, little can be said at the present time. Since protoproteoses, deuteroproteoses, and peptone are split off on digestion with pepsin, the molecule is evidently larger than that of any of the albumoses. The well-purified substance seems to be free from phosphorus, and hence contains no nucleins ; but it contains considerable sulphur (generally between 1 and 2 per cent.), which is readily split off. Like casein, it contains no hetero-group (lack of heteroproteoses on digestion), but differs in containing a carbohydrate group (in small amount) and in the absence of phosphorus. On hydrolysis Magnus-Levy¹ obtained glutaminic acid, tyrosin, and

¹ Zeit. physiol. Chem., 1900 (30), 200.

leucin, but no glycocoll. He found the nitrogen distributed as follows: amid-nitrogen, 9.9 per cent.; humin-nitrogen, 9.8 per cent.; diamino-nitrogen, 6.4 per cent.—which last was composed of: histidin, 0.9 per cent.; arginin, 2.4 per cent.; lysin, 3.0 per cent. Attempts to prove the identity of the body by the precipitin reaction have failed.¹

Occurrence of "Myelopathic Albumosuria."—At the present time (1906) there are between forty and fifty authenticated cases of "myelopathic albumosuria" in the literature, but the number is rapidly increasing as the general appreciation of its characteristics is widening. Not all cases of multiple myeloma show the presence of Bence-Jones proteid in the urine however.² It is still uncertain as to whether this substance is produced specifically in multiple myeloma or is present occasionally in other conditions. Multiple bone involvement by other tumors does not cause "albumosuria."³ There is no evidence that it occurs in the normal body, even in the bone-marrow, or that it is produced as a step in the splitting of any form of proteids. A few cases of supposed osteomalacia have been reported, with the Bence-Jones body in the urine, but on more careful investigation these seem to have been unrecognized myelomas (*e. g.*, the cases of Bence-Jones and of Jochmann and Schumm). Similarly the case reported by Askanazy as leukemia with Bence-Jones proteid in the urine, on reëxamination was found to be multiple myeloma. Coriat⁴ describes a substance found in a pleuritic fluid which gave the reactions of the Bence-Jones body, and he believes that it may have been formed from serum-globulin through the digestive action of the leucocytes or bacteria. Zuelzer reports finding the same body in the urine of a dog poisoned with pyridin.⁵

Origin of the Proteid.—As to the place of formation of this peculiar proteid, there is much diversity of opinion. Magnus-Levy advanced against the idea that it is formed by the tumor cells the following arguments: In the urine of myeloma patients are excreted great quantities of the proteid,—as much as 30 to 70 grams per day,—whereas the total amount of proteid in all the tumor tissue in the body seldom exceeds,

¹ Rostoski, Verh. der Phys. Med. Gesell., Würzburg, 1902 (35), 30; Münch. med. Woch., 1902 (49), 740.

² See Collins, Med. Record, 1905 (67), 641.

³ A case of this kind has, however, recently been described by Oerum (Ugeskrift f. Lager, 1904, No. 24), in which the bone tumors were multiple metastases of a gastric carcinoma.

⁴ Amer. Jour. Med. Sci., 1903 (126), 631.

⁵ Wohlgemuth (Arb. a. d. Path. Inst. zu Berlin, Festschrift, 1906, p. 627) states that normal human bone marrow may contain true albumoses.

or, indeed, equals this quantity. It seems improbable that so little tumor tissue can form so much urinary proteid, and Magnus-Levy suggests that it must come from the food proteids as a result of altered proteid metabolism. Against this view, however, are the following facts: (1) The Bence-Jones body has been found (but not constantly) in the myeloma tissue, but not in other organs or tissues; (2) the quantity in the urine is not dependent upon diet;¹ (3) it is associated only with this form of tumor. Simon considers it probable that the proteid is formed from serum-globulin, perhaps by an enzymatic action of the tumor cells, and once formed, it is rapidly eliminated by the kidneys, as are all foreign proteids.

¹ See Allard and Weber, *Deut. med. Woch.*, 1906 (32), 1251. Voit and Salvendi (*Münch. med. Woch.*, 1904 (51), 1281), however, report a case in which diet modified the elimination of the "albumose."

CHAPTER XVIII

PATHOLOGICAL CONDITIONS DUE TO, OR ASSOCIATED WITH, ABNORMALITIES IN METABOLISM, INCLUDING AUTOINTOXICATION

DURING the course of metabolism innumerable organic compounds are formed, some of which are of a more or less poisonous nature. As long as the body is in a normal condition, these injurious substances are kept from accumulating in sufficient quantities to do harm; this is accomplished in one of the following ways: (1) Elimination from the body in the urine, feces, etc.; (2) combination with other substances into harmless, or relatively harmless, compounds; (3) chemical alteration into compounds that are non-toxic or relatively innocuous. Therefore a harmful accumulation of metabolic products may be the result of any one of the following conditions:

(1) Failure of elimination because of abnormal conditions in the eliminating organs; *e. g.*, uremia.

(2) Failure of neutralization by chemical combination, presumably due to abnormalities in the organs or tissues through whose activities the neutralization is normally accomplished; *e. g.*, diseases of the liver.

(3) Failure in the chemical transformation of the metabolic products; this may result either from abnormalities in the functioning tissues, or through a checking of the normal steps of metabolism by the failure of elimination of the end-products.

(4) Excessive formation of certain normal products of metabolism; *e. g.*, hyperactivity of the thyroid.

(5) Production of abnormal toxic chemical substances; *e. g.*, the intoxication following superficial burns.

Numerous classifications of autointoxication have been proposed by various authors, some excluding from the causes of autointoxication all but the products of metabolism within the blood and tissues of the body, as has been done in the preceding consideration; many including intoxications caused by the products of gastro-intestinal fermentation and putrefaction; and still others (v. Jaksch) including even the intoxications produced by bacterial invasion of the body.¹ It is

¹ See résumé by Weintraud, *Ergeb. der Path.*, 1897 (4), 1.

extremely difficult to draw the line as to just what should be included under the term autointoxication, and particularly difficult to decide the proper placing of the intoxication resulting from fecal retention and from processes of decomposition in the alimentary canal. For example, the poisoning following the eating of partially decomposed canned food could not be looked upon as an autointoxication, and yet there is no fundamental difference whether the decomposition occurs, as in this case, before the food enters the body, or whether it occurs in the intestinal tract because of abnormal bacteriological or anatomical conditions. On the other hand, since many of the obnoxious products of metabolism are eliminated through the bowels, failure of elimination through this channel may lead to a true autointoxication as much as may deficient renal elimination. On the whole, it seems best to restrict the term autointoxication, as far as possible, to the disturbances produced by products of metabolism that have been formed within the tissues of the body (*intermediary metabolism*), considering as a distinct but related subject gastro-intestinal autointoxication.

In the discussion of autointoxication from the standpoint of chemical pathology, we are interested particularly in the chemical nature of the substances that cause the intoxication, and in the chemical processes by which their action is kept at a minimum, rather than in the clinical features or anatomical results that may be produced. Unfortunately, in but a few instances have the exact chemical substances causing these intoxications been accurately determined, probably because in most cases not one but a number of poisonous substances are present; and, furthermore, we do not always know exactly when a certain disease is to be ascribed to autointoxication, nor can we always determine that the cause of a certain intoxication lies in an abnormality in metabolism and not in an infection of hidden nature. It is, therefore, quite impossible, with the uncertain information available at this time, to consider autointoxication in a systematic way, and we must limit ourselves to a consideration of certain pathological conditions in which there appears to be an element of abnormal metabolism with resulting intoxication. In some cases this intoxication is a prominent feature of the disorder, in others it is subordinate to other manifestations of the disease; and, finally, we may have marked alterations in metabolism without evidences of disturbance of health (*e. g.*, cystinuria, alkaptonuria).

Of the autointoxications due to the retention of poisonous products of metabolism that should be excreted from the body,

first in order of importance stand uremia and cholemia (the latter has already been considered in connection with the discussion of Icterus, Chap. xvi). Of apparently less significance are autointoxications due to failure of elimination of gaseous metabolic products by the lungs, and failure of the excretory functions of the skin.

UREMIA¹

The cause or causes of the severe, often fatal, intoxication that may occur when the outflow of urine is completely checked, or when it is qualitatively and quantitatively altered for long periods of time, have not yet been definitely determined. As the kidney seems to be the chief organ for the removal of the products of nitrogenous metabolism, it is naturally assumed that uremia is the result of a retention of these products, but as yet it has not been ascertained which of the many products is responsible, and, indeed, there are very good reasons for questioning if the substances present in normal urine do or can cause uremia when their elimination by the kidney is defective. There is no question but that the urine contains toxic substances. Among them are the salts of potassium, which, however, cannot alone explain all the urinary toxicity, for the symptoms produced by the injection of urine are different from those produced by potassium salts, and it has been found that the inorganic constituents (ash) of urine are less poisonous than the entire urine. Furthermore, toxic mixtures of organic, ash-free substances have been obtained from normal urine.² Of the known normal constituents of the urine there are few, however, that are toxic to any considerable degree, and these occur in but very small quantities. Urea is generally considered as almost absolutely non-toxic,³ the animal body withstanding injection of large quantities without appreciable injury. Uric acid, the purin bases, hippuric acid, creatinin, and the urinary pigments are all possessed of very slight toxicity, and their effects do not explain the manifestations of uremia. Injections of urine into animals may cause more or less disturbance, but it is different, on the whole, from the manifestations of uremia. (The experiments of Bouchard and his school present such serious errors

¹ General résumé with literature by: Honigmann, *Ergeb. der Pathol.*, 1894 (Bd. 1, Abt. 2), 639; 1902 (8), 549; Ascoli, *Vorlesungen über Urämie*, Jena, 1903.

² See Dresbach, *Jour. Exp. Med.*, 1900 (5), 315.

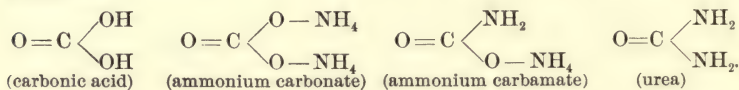
³ Herter ascribes more importance to urea in uremia than do many other authors (see Johns Hopkins Hospital Reports, 1900 (9), 69).

of technique and interpretation that they are now largely disregarded.)

For these and other reasons, it is generally considered that the intoxication of uremia is not due solely or chiefly to the substances that are normally eliminated in the urine,¹ but rather to more toxic antecedents of the nitrogenous constituents of the urine. Urea represents but the final product of a long series of reactions by which the huge proteid molecule is broken up into its "building-stones," the various amino-acids, and these in turn are decomposed in such a way that their NH_2 groups are combined with carbonic acid² and eliminated as the diamido-

compound of carbonic acid, namely urea, $\text{O}=\text{C}\begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix}$. We

know that the liver is able to accomplish the conversion of amino-acids to urea, for it has been experimentally shown that if leucin and glycocoll are passed through the vessels of the isolated liver they disappear in part, while an increased amount of urea escapes from the hepatic veins. It is probable that the liver is the chief site of urea formation, but it is also probable that urea can be formed in other organs. We do not know, however, the intermediate steps by which the amino-acids of the proteid molecule are converted into urea. It has been repeatedly shown that urea can be formed from ammonium salts of organic acids (including ammonium carbonate), and ammonia is a constant product of autolysis, being characteristically more abundant as a product of autolytic proteolysis than as a product of tryptic proteolysis; therefore, one of the antecedents of urea is probably ammonia, which is somewhat toxic and especially hemolytic.³ Another antecedent of urea is ammonium carbamate, which stands in structure intermediate between urea and ammonium carbonate, as shown by the following graphic formulæ:



That ammonium carbamate is probably an important precursor of urea has been shown particularly through the results of studies of dogs with Eck's fistula,⁴ which consists of a fistula

¹ See Bradford, Practitioner, 1901 (67), 507.

² Arginin alone of all the amino-acids is known to split off urea directly from its molecule.

³ Concerning the toxicity of ammonium salts see Rachford and Crane, Medical News, 1902 (81), 778.

⁴ See Hahn, Massen, Nencki, and Pawlow, Arch. f. exp. Path. u. Pharm., 1893 (32), 161.

between the portal vein and the inferior vena cava, the blood from the portal system then passing directly into the general circulation without first passing through the liver. In such animals the urine becomes poor in urea and relatively rich in ammonium carbamate. At the same time, the dogs show severe symptoms of intoxication from which they die, and which are similar to the symptoms that follow intravenous injection of ammonium carbamate. Ammonium carbamate, being a substance of considerable toxicity¹ when free in the blood, it has, therefore, been quite widely considered that it may be an important factor in the production of uremic symptoms. On the other hand, it seems most probable that the condition of uremia does not depend upon one but upon many various and varying substances. Clinically the symptoms of uremia in different cases are widely different; thus, if uremia is due to complete suppression of urine through mechanical obstruction, the symptoms are quite different from those observed in the uremia following a chronic nephritis; drowsiness, weakness of heart action, and syncope being the chief manifestations of obstructive uremia, the convulsions and other manifestations of nervous irritation characteristic of uremia in chronic nephritis being absent.

Chemical Change in Uremia.—The attempts to isolate from the blood and organs of uremic patients or animals toxic substances that explain the manifestations of uremia have thus far failed.² That there is an actual retention of organic substances in the blood in uremia is shown conclusively, however, by the studies of the physicochemical properties of the blood. It has been repeatedly found that in uremia the *freezing-point* of the blood is reduced markedly below the normal;³ instead of the normal depression of 0.55° – 0.57° the freezing-point is usually reduced more than -0.60° , and sometimes as much as $-.75^{\circ}$, which shows that the number of molecules in the blood is increased.⁴ At the same time, the *electrical conductivity* may not be at all increased (Bickel⁵), but may even be reduced; and as the electrical conductivity of the blood depends upon the number of dissociable molecules, chiefly inorganic salts, these are evidently not increased. Therefore, the increased number of molecules must represent

¹ See Bickel, "Exp. Untersuch. über Cholaemie," Wiesbaden, 1900.

² See Couvée, Zeit. klin. Med., 1904 (54), 311.

³ See Tieken, Amer. Med., 1905 (10), pp. 393, 567, and 822; complete literature.

⁴ See table of freezing points of blood and effusions on page 298.

⁵ Deut. med. Woch., 1902 (28), 501.

an excess of organic molecules that dissociate but little if at all, and hence are not conductors of electricity. Some authors, indeed, have ascribed uremia to the increased osmotic pressure of the blood from the retained molecules, but this is improbable, according to Strauss,¹ who found that a marked increase in molecular concentration may occur without uremia, and that we may have a severe uremia without increased osmotic pressure.²

That organic nitrogenous bodies accumulate in the blood in nephritis has been repeatedly demonstrated. Strauss found that the non-proteid nitrogen of the blood, which normally amounts to 20–35 mg. per 100 c.c. of blood, shows a slight increase in chronic parenchymatous nephritis, to about 40 mg.; and in interstitial nephritis, a large increase, the total amount averaging 85 mg., being especially high when uremia is present. Urea is demonstrably increased under the same conditions, as also is the ammonia nitrogen, especially in uremia. Erben³ has studied the variations in the normal components of the blood during nephritis, and found the albumin generally decreased in proportion to the globulin, especially in the case of parenchymatous nephritis; lecithin and calcium are also decreased. The decrease in red corpuscles and hemoglobin in nephritis is a well-known feature. By the precipitin reaction it has been shown that the globulin of nephritic urine is derived from the serum, and not directly from the proteids of the food. Rumpf⁴ has analyzed the organs as well as the blood in nephritis, and found a distinct retention of organic substances in both the blood and organs; sodium chloride is usually increased, as also are the other inorganic salts, which are probably partly bound in organic combination with the tissues. (See "Retention of Chlorides in Edema," p. 293.) The reduction of the alkalinity of the blood, observed by v. Jaksch and others in uremia, is attributed by Gottheiner⁵ to the presence of abnormally large quantities of lactic acid in the blood. Orłowski⁶ found that an accumulation of acids occurs in uremia, but not until just before death, and, therefore, the reduction of blood alkalinity is not the cause, but an accompaniment of the uremia. Further-

¹ Die chronischen Nierenentzündungen, etc., Berlin, 1902.

² Stern (Med. Record, 1903 (63), 121) notes that the electrical conductivity is reduced by the presence of excessive quantities of non-electrolytes in uremia, and regards this lowered conductivity as a factor of some possible importance.

³ Zeit. klin. Med., 1903 (50), 441; 1905 (57), 39.

⁴ Münch. med. Woch., 1905 (52), 393.

⁵ Zeit. klin. Med., 1897 (33), 315.

⁶ Zentr. f. Stoffwechsel u. Verdauungskr., 1902 (3), 123.

more, in other diseases a corresponding or greater reduction in alkalinity may occur without uremia. The development of this terminal acidity, together with the finding of albumose in the blood of a nephritic by Schumm,¹ suggests the probability of active autolytic processes occurring in uremia. Neuberg and Strauss² have also found glycocoll in considerable quantities (1.5 per mille) in the blood-serum of a uremic patient and in the blood of nephrectomized rabbits.

The Cause of Uremia.—Putting all the known facts together, we find the weight of evidence indicating that uremia is due to poisoning with organic substances, probably antecedents of urea, but of unknown nature. The poison or poisons may be sufficiently concentrated to cause structural alterations in the cortical ganglion-cells (chromatolysis) which have been repeatedly found in uremia. As yet, however, we are completely in the dark as to whether the substances causing the uremia are such well-known antecedents of urea as ammonium carbamate and other ammonium salts, or some quite specific and unfamiliar nitrogenous substances which arise in the cells as the result of the action of accumulated decomposition-products of the proteids. To account for an accumulation of the antecedents of urea we do not need to assume a perversion of metabolism as the cause, if we appreciate that the various reactions of metabolism, being due to catalytic agents, go on to the point of establishing a chemical equilibrium. If for any cause the kidneys cannot excrete all the urea formed, its accumulation in the blood and tissues will necessarily lead to a blocking of the steps of urea formation, and a corresponding accumulation of the antecedents of urea in the body. On the other hand, it is to be borne in mind that the decrease in elimination of nitrogen in nephritis is not so great as is ordinarily assumed, the popular error being due to the fact that most clinical estimations of urinary nitrogen are based on the determination of the urea alone. In nephritis the urea may constitute a much smaller proportion of the total urinary nitrogen than in health, on account of the relatively greater proportion of nitrogen eliminated in other forms. According to numerous observers, particularly the Italians, the proportion of these intermediary nitrogenous bodies may be increased in the blood, even when the urinary nitrogen is normal in amount, and if this statement is correct, then presumably abnormal metabolism, rather than defective renal elimination, is primary; in which case the renal lesions

¹ Hofmeister's Beitr., 1903 (4), 453.

² Berl. klin. Woch., 1906 (43), 258.

may have been produced secondarily by the products of the abnormal metabolism.

While admitting the preponderating importance of toxic organic substances as the cause of uremia, we cannot dismiss as altogether unimportant the changes in osmotic pressure in the blood and tissue fluids, even although it has been shown by Strauss and others that there is no constant relation between the osmotic pressure of the blood and the uremic attack. It still seems quite possible that the hyperosmotic condition of the fluids in the brain is the determining factor in some uremic attacks. Neither can we entirely dismiss the edema of the brain and meninges that is associated with this hypertonicity, from the possible factors in the production of uremia. The "wet brain" of the uremic is too frequent an autopsy finding to be without importance, and clinicians have repeatedly noted a favorable effect from spinal puncture in uremia, following the escape of a fluid under apparently abnormally high pressure.¹

The Internal Secretion of the Kidney.—Another possible factor in uremia is the hypothetical internal secretion of the kidney. Bradford,² through an extensive experimental study of the effects of partial resection of the kidney tissue, found that if three-quarters of the total kidney tissue be removed (in dogs) death occurs with profound tissue wasting and asthenia, which is associated with an elimination of more urea and water than a normal animal passes with two complete kidneys. The fragment of kidney left is able to excrete amounts of urea far larger than those usually excreted, as is shown by giving the animals considerable quantities of an exclusively meat diet. Of particular importance is the fact that the amount of nitrogenous extractives (non-proteid nitrogen) in the blood and tissues, especially the muscles, is much greater than in normal animals, even when the nitrogen excretion is above normal; which indicates that loss of renal tissue results in excessive proteid katabolism, and suggests that the kidneys have an important function in regulating proteid metabolism through the production of an internal secretion with *inhibitory effect on metabolism*. In accordance with this, Vitzou³ claims to have demonstrated that the blood from the renal vein contains substances which decidedly reduce the severity of uremia in experimental animals. If this contention is true, there exists the possibility that in interstitial nephritis the loss of renal tissue may cause a deficiency in an

¹ See Willson, Jour. Amer. Med. Assoc., 1904 (43), 1019.

² Jour. of Physiol., 1899 (23), 415.

³ Jour. Phys. et Path. Gén., 1901 (3), 901 and 926.

internal secretion which depresses proteid katabolism, and thus leads to an excessive formation of nitrogenous substances in the tissues and their accumulation in the blood.

ECLAMPSIA¹

In many respects eclampsia resembles uremia; so much so, indeed, that Frerichs and others have referred to eclampsia as "puerperal uremia." Considering it as a simple uremia occurring in pregnancy, uremia and eclampsia have in common the constant occurrence of renal disturbance with albuminuria and decreased elimination of urea, and also violent convulsions and profound coma terminating in death. On the other hand, eclampsia differs greatly from uremia in the anatomical changes observed in the organs of the body other than the kidneys; these are of such a nature that in some cases it becomes difficult to distinguish eclampsia from acute yellow atrophy of the liver, while in other cases the picture resembles that of a profound bacterial intoxication, so that numerous authors have urged that eclampsia is the result of a bacterial infection. At the present time the cause of puerperal eclampsia is quite unknown, but there is a decided tendency to assume that poisonous substances are developed in the placenta or fetus, or are formed in the body as a reaction of the maternal organism to the foreign fetal elements. These theories will be discussed after considering the known facts concerning the chemical changes of the disease that have been reported by various observers.

Chemical Changes in Eclampsia.—*Urinary changes* are practically invariably present, and usually they are profound, although there are no known characteristic qualitative or quantitative differences from the urinary changes of puerperal albuminuria without eclampsia. Proteids are abundant, including a large proportion of globulin, decreasing rapidly after delivery as a rule. The urea is usually very low, but generally increases with great rapidity after delivery, until two or three times the normal amount is passed per day; as urea and ammonia do not seem to be increased in the blood, this indicates that during eclampsia there is an accumulation of the precursors of urea in the system (Sikes). There is an excessive elimination of nitrogen in the form of ammonia, which seems to be due to the formation of abnormal quantities of sarcolactic and

¹ A thorough review of the literature is given by Sikes in *The Practitioner*, 1905 (74), pp. 478 and 642, with complete bibliography. Only more recent references will generally be cited in the text.

other organic acids in the body, which are combined with ammonia in the blood and eliminated in the urine.¹ This fact has led many to look with favor upon the idea that eclampsia is due to an acid intoxication. Other nitrogenous urinary constituents may also be increased, so that the relative proportion of nitrogen eliminated as urea is often greatly reduced. The proportion of sulphur eliminated in an unoxidized form, as compared with that eliminated as SO_4 , is much greater than normal. These findings all indicate that oxidation within the body is impaired.

Numerous writers have studied the *toxicity of the urine* in eclampsia, but the earlier investigations were conducted in such a manner that the results are practically worthless. More recent studies by Volhard, Schumacher, and Van der Bergh yield no evidence that the urine shows any actual differences in toxicity whether from normal, pregnant, or eclamptic women.

Analyses of the blood have given widely differing results, some finding an increase in urea, while others have failed to observe such increase (the latter including the more recent observations). Likewise the statements concerning the quantity of ammonia in the blood are at variance, Zweifel holding that neither urea nor ammonia is increased. The decrease in the alkalinity of the blood observed by Zangemeister and others has been ascribed to the formation of sarcolactic acid by Zweifel,² who failed, however, to find an excess of CO_2 , or to detect oxybutyric acid or oxalic acid in the blood. As to the blood proteids, fibrin has been found increased by Kolman and by Dienst, while Schmidt found a relative increase in the globulin. Sikes concludes that the statements to be found in the literature concerning the toxicity of the blood in eclampsia leave nothing proved concerning this point.

Theories as to Etiology.—The anatomical changes of eclampsia are such as to leave little or no room for doubt that there is a severe intoxication with poisons that have a markedly toxic effect upon all the organs of the body, thus differing from the toxic materials at work in uremia, which seem to affect chiefly the central nervous system. Repeated bacteriological and histological studies have failed to demonstrate that infection with either vegetable or animal parasites is the cause, and clinical observations do not support such a hypothesis. The association of the condition with pregnancy, and particularly the rapid improvement that follows the removal of the contents

¹ See Zweifel and Lockmann, Münch. med. Woch., 1906 (53), 297.

² Arch. f. Gyn., 1905 (76), 537.

of the uterus, almost compels us to admit that the causative agent is produced by the fetus or the placenta. Some investigators (Politi, Liepmann) believe that they have found a greater degree of toxicity in extracts from the placentas from eclamptic than from normal women. We have no approximate ideas as to the nature of the supposed toxic substances, except that Zweifel,¹ who is the leading exponent of the idea that eclampsia is an acid intoxication, believes that the fetus produces abnormal quantities of *lactic acid* which is the cause of the maternal intoxication. In support of this view he reports the finding in eclampsia of lactic acid in blood from the umbilical vein in much greater quantities than in the maternal blood, and in still greater quantities in the placenta. It seems improbable, however, that the severe anatomical changes and the convulsive manifestations, so different from the conditions observed in ordinary acid intoxications, can be due to sarcolactic acid alone, especially when in such minute quantities as it is found in the blood of eclamptics.

The Placenta as a Source of Intoxication.—Histologists having frequently observed placental cells in the blood and vessels of eclamptic patients, it has been suggested that multiple *capillary emboli of placental cells*, detached from the chorionic villi and forced into the placental circulation, cause the manifestations of the disease; this theory is entirely inadequate, however, to explain all the features of eclampsia. Related to this hypothesis is the idea that the placental tissues, being foreign to the maternal organism in so far as they are derived from the ovum, give rise to the production of antibodies (*syncytiolysins*) by the mother, which are toxic for pregnant animals (Ascoli), and which may have to do with eclampsia in some unknown way. In any case, antiserum for placental tissue has been repeatedly prepared (Weichardt, Scholten and Veit,² Opitz³). Possibly the foreign tissues are toxic to the maternal organism, or form toxic substances during their solution (Weichardt), and a failure to develop such antibodies as have been obtained experimentally leaves the mother unprotected from these toxic substances. Up to the present time, however, this phase of the study of the pathogenesis of eclampsia has yielded little besides interesting but contradictory hypotheses.⁴

Liepmann⁵ has reported the finding of a considerable degree of toxicity in eclamptic placentas; the poisonous substance,

¹ *Loc. cit.*

² *Zeit. f. Geb. u. Gyn.*, 1903 (49), 210.

³ *Deut. med. Woch.*, 1903 (29), 597.

⁴ See review by Wormser, *Münch. med. Woch.*, 1904 (51), 7 and 2285.

⁵ *Münch. med. Woch.*, 1905 (52), 687 and 2484.

which is extremely labile, being firmly united to the proteid molecule, and as yet not successfully isolated.

The Fetus as a Source of Intoxication.—A reasonable view of the cause of eclampsia is that it is initiated by the excessive products of metabolism thrown into the blood of the mother, both from the fetus and from her own overactive tissues; these cause injury to the kidneys, leading to a further retention, or injure the liver so that the normal metabolic processes of that organ (particularly oxidation) cannot be carried on; or, perhaps more often, both liver and kidney as well as other organs are injured. In this way a vicious circle is established which rapidly leads to an overwhelming of the maternal system with toxic products derived from both her own and the fetal tissues. It must be admitted, however, that the rapid improvement that so often follows removal of the products of conception indicates strongly that the poisonous substances arise chiefly, if not exclusively, in the fetus or the placenta. But, as Liepmann points out, the child shows relatively little evidence of intoxication, while, on the other hand, eclampsia may develop *after* delivery of the fetus, which facts speak in favor of the place of the origin of the poison being the placenta and not the fetus. Especially important in this connection is the observation of a case of eclampsia by Hitschmann¹ in a patient with a hydatid mole and no fetus.²

The Thyroid in Eclampsia.—In view of the mystery surrounding the cause and effect of the enlargement of the thyroid during pregnancy, it is not strange that the suggestion has been made that the enlargement is for the purpose of neutralizing the excessive amounts of toxic materials in the maternal blood, and that failure of this enlargement is responsible for eclampsia. In support of this idea Lange³ states that absence of the normal thyroid enlargement is usual in eclampsia, and Fruhins-holz and Jeandelize⁴ note the frequency of eclampsia in myxedematous women.

Summary.—Most of the facts at hand speak against the idea that one definite chemical substance is responsible for the anatomical changes and symptomatic manifestations of eclampsia. More probably there are present not only the

¹ Cent. f. Gyn., 1904 (28), 1089.

² Dienst (Cent. f. Gyn., 1905 (29), 353) has advanced the proposition that in eclampsia there is a mixture of the heterogeneous fetal blood with that of the mother, based on the finding of direct communication between the maternal and fetal circulation in eclampsia.

³ Zeit. f. Geb. u. Gyn., 1899 (40), 34.

⁴ Presse Méd., 1902 (10), 1023.

poisonous substances that initiate the tissue changes, but also toxic substances that accumulate because of the disorganization of the liver and kidney cells, and which are possibly similar to the toxic substances most prominent in uremia and in acute yellow atrophy, for eclampsia seems to stand intermediate between these two diseases, encroaching upon the characteristics of each. Acid intoxication, which undoubtedly exists to a greater or less degree in some cases of eclampsia, is probably not usually the chief cause of the clinical manifestations of the disease. The finding of minute quantities of lactic acid in the blood, urine, and in the cerebrospinal fluid (Füth and Lockemann¹) is perhaps not of great significance, for, as Wolf² rightly insists, similar amounts may be found in other conditions associated with convulsions and partial asphyxia, or in partial starvation, such as results from the vomiting of pregnancy. To be sure, Zweifel states that lactic acid may be found in the urine and blood before the eclamptic seizures have appeared, but, in any case, the anatomical changes and clinical manifestations cannot be explained as due to the action of the trifling quantities of sarcolactic acid found in the blood of these patients. The excretion of these organic acids, as well as the large proportion of unoxidized sulphur in the urine, indicates that incomplete oxidation is an important feature of eclampsia, and under such conditions a large number of imperfectly known toxic substances may accumulate in the blood and tissues. The defective oxidation is probably the result of the injury to the liver-cells, which have such a prominent oxidizing function.

ACUTE YELLOW ATROPHY OF THE LIVER

In this condition there is presented a striking picture of autolysis, in that a large parenchymatous organ undergoes a rapid reduction of size because of a solution of its structural elements, while at the same time products of proteid digestion (leucin, tyrosin, etc.) appear free in the liver, the blood, and the urine. Because of these prominent features and their relation to the questions of metabolism in general, and the function of the liver in particular, acute yellow atrophy of the liver has been the object of much greater interest and investigation than its clinical importance would warrant, for it is a rare disease, there probably being but about 500 cases reported in the literature, according to Best's figures.³

¹ *Cent. f. Gyn.*, 1906, p. 41.

² *New York Med. Jour.*, 1906 (83), 813.

³ Thesis, University of Chicago, 1903.

The etiology of the disease is quite unknown, but it is very probably not a specific one, for we find that numerous forms of intoxication may lead to a condition closely resembling acute yellow atrophy,¹ particularly phosphorus poisoning, chloroform poisoning, puerperal eclampsia, and some cases of septicemia (especially with the streptococcus²), arsenic poisoning, and mushroom poisoning. It seems probable that any poison which does not directly cause death, but which causes a severe injury to the liver-cells without at the same time destroying the autolytic enzymes, so that the cells die and undergo rapid autolysis, may produce a condition identical with or similar to acute yellow atrophy (Wells and Bassoe³). In the typical cases of the disease, of "idiopathic" origin, the poisonous agent probably comes from the alimentary canal, as indicated by a preliminary period of gastro-intestinal disturbance that usually precedes the onset of the disease, and secondly by the fact that the liver seems to receive the chief effect of the poison. Whether these hypothetical poisons are produced by abnormal fermentation and putrefaction in the alimentary tract, or by a specific organism elaborating its poison in this location, is quite unknown. Bacteriological studies of the disease have so far given inconstant and non-instructive results. In the countries where phosphorus poisoning is common (especially Austria) there has been found much difficulty in distinguishing in many cases the results of phosphorus poisoning from acute yellow atrophy of the liver, and many have contended that there is no real difference; *i. e.*, that phosphorus, as well as unknown poisons, may cause acute yellow atrophy. The present trend of opinion, however, seems to favor the view that there is a primary liver atrophy which is different from that caused by phosphorus or other known poisons in several essential respects.⁴

Phosphorus Poisoning.—*Between phosphorus poisoning and "primary" hepatic atrophy the following chief differences may be discerned:* Phosphorus produces a general injurious effect upon all the organs of the body, the liver merely showing the most marked anatomical changes, which at first consist of a fatty metamorphosis of the liver, due to migration of the body fat

¹ It is to be borne in mind that the color is yellow only during the earlier stages, "red atrophy," occurring later, but the name acute "yellow atrophy" has come through usage to apply to the disease as a whole.

² Babes, *Ann. Inst. Path. Bucarest*, vol. 6.

³ *Jour. Amer. Med. Assoc.*, 1904 (44), 685.

⁴ See Anschütz, *Arb. a. d. Path. Inst. Tübingen*, 1902 (3), 230; Paltauf, *Verh. Deut. Path. Gesell.*, 1903 (5), 91; Riess, *Berl. klin. Woch.*, 1905 (42), No. 44a, p. 54.

from the fat deposits into the injured cells (Rosenfeld, Taylor); subsequently the liver-cells disintegrate, the cytoplasm being affected before the nucleus, and the liver may become smaller than normal, although it is usually enlarged because of the fat deposition. Typical acute yellow atrophy is characterized by an early necrosis of a large proportion of the liver-cells, the nucleus becoming unstainable while the cytoplasm is still little altered in appearance, and fatty changes play a subordinate rôle or are absent. As Anschütz says, the poison seems to strike at the life of the cell, its nucleus, while phosphorus attacks the cytoplasm. Furthermore, the poison of yellow atrophy seems to be very specific, for it attacks the other organs of the body almost not at all, and within the liver it affects only the hepatic cells proper, while the bile-duct epithelium and the stroma cells are so little injured that they are able to proliferate greatly, this proliferation being a prominent feature. There are also clinical and chemical differences that will be discussed later, but yet, on the whole, the resemblances of yellow atrophy and phosphorus poisoning are so great that we have obtained much information concerning the former by means of experimental studies of phosphorus poisoning.

Delayed Chloroform Poisoning.—After *chloroform narcosis* there occasionally develops a severe intoxication, with clinical and anatomical findings very similar to acute yellow atrophy and phosphorus poisoning;¹ in point of the fatty changes the cases usually resemble more the phosphorus poisoning. Some cases of puerperal eclampsia also present such profound liver changes that they are distinguished as eclampsia chiefly on the basis of the convulsive manifestations, rather than on the ground of anatomical changes. So, too, the hepatic changes in certain septicemias may resemble those of acute yellow atrophy to a greater or less degree.

Summary of Views on Etiology.—From a review of the literature and the study of a few cases, the writer has reached the following understanding of the condition described as acute yellow atrophy of the liver: The "atrophy" is due entirely to autolysis of necrotic liver-cells by their own enzymes. In the most typical cases of "primary" or "idiopathic" yellow atrophy we have to do with a poison having a very specific effect on the liver-cells, which destroys their "life" (*i. e.*, stops synthetic activities) without injuring their intracellular proteolytic enzymes, and consequently autolysis occurs; as the poison

¹ Complete review and literature by Bevan and Favill, Jour. Amer. Med. Assoc., 1905 (45), 691.

affects other organs but little, the necrosis and autolysis continue until there is so much loss of liver function that systemic poisoning results from the hepatic insufficiency and from the resulting accumulation of poisonous products of incomplete metabolism. The patient dies from this poisoning,¹ and the liver is found at autopsy to have decreased by from one-third to one-half or more in its volume. This great change would not be possible if the poisons affected the heart, kidneys, or brain as much as they do the liver structure, which is probably the reason that phosphorus, bacterial poisons, snake poisons, and other poisons that destroy liver-cells do not ordinarily produce the typical picture of liver atrophy. When these poisons affect the liver more and the other tissues less, we approach the condition of acute yellow atrophy; *e. g.*, if the dose of phosphorus is not so great as to kill the patient through injury of other more vital organs, after a few days the necrosed liver-cells undergo autolysis, and if enough liver-cells have been destroyed, hepatic insufficiency may cause death, with the finding of an anatomical condition in the liver that can be properly designated as acute atrophy. Hence it is possible for many poisons to cause this condition under certain circumstances, and there seem to be certain unknown poisons (probably of intestinal origin²) that are of such a nature that they cause specifically acute hepatic atrophy. The above hypothesis seems to explain all the known facts concerning this disease. That phosphorus, chloroform, and some other poisons lead particularly to fatty changes may, perhaps, be due to their acting especially upon the oxidizing enzymes, leaving the autolytic enzymes and the lipase free to digest the cell and to form fat.³ That it is particularly the oxidizing enzymes that are attacked is well shown by the chemical findings, and also by Loewy's⁴ observation that in poisoning with CNH, which acts by impairing oxidation, the alterations in proteid metabolism are very similar to those of phosphorus poisoning.⁵

¹ The mortality of cases sufficiently typical to be diagnosed antemortem is estimated by Rondaky (Roussky Vrach, Oct. 28, 1900) at 97 to 98 per cent. Concerning the regenerative changes in the cases which recover, see Yamasaki (Zeit. f. Heilk., Path. Abt., 1903 (24), 248).

² See Carbone, Riforma Med., 1902 (1), 687 and 698.

³ Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

⁴ Cent. f. Physiol., 1906 (19), 23.

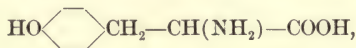
⁵ The hypothesis suggested by Quincke (Nothnagel's Handbook, 1899, vol. 18, p. 307) that possibly regurgitation of pancreatic juice up the bile-ducts might be responsible for the degenerative changes in the liver, is contradicted by the fact that the bile pressure is greater than the pancreatic juice pressure, and that the bile-ducts and peripheral portions of the lobules are least affected. Nor could Best prove that trypsin injected into the liver by way of the bile-ducts is able to cause such changes. (See Wells and Bassoe, *loc. cit.*)

CHEMICAL CHANGES OF ACUTE YELLOW ATROPHY

The Urine.—Most striking, and long regarded as pathognomonic, is the presence of *leucin* and *tyrosin* in the urine, first described by Frerichs. While we now know that these and other amino-acids may occur in the urine in any conditions where there is a great breaking down of tissue within the body, yet it is true that in no other condition are they found so abundantly as in acute hepatic atrophy (as high as 1.5 gm. of tyrosin per diem has been found¹). They are nearly constantly present (in thirteen out of fourteen cases studied by Riess²), tyrosin being usually the more abundant. *Deutero-proteose* is also frequently (but not constantly) found, as further evidence of abnormal proteid splitting.³ Uric acid and other purins are often somewhat, but not characteristically, increased, probably resulting from the nuclear destruction in the liver. The total elimination of nitrogen is increased⁴ (particularly if the scanty intake is considered), and the proportion that appears as urea is decreased, largely because of the presence of much ammonia, part of which, at least, is eliminated combined with organic acids. Chief of these acids is *sarcolactic acid*, but of particular interest is the appearance of *oxymandelic acid*,



which is apparently derived from tyrosin,



by the splitting out of the NH_2 group, the benzene nucleus failing to be completely oxidized, as is normally the case. It is evident from the urinary findings, therefore, that oxidation is decreased, which is presumably because of the destruction of liver tissue with its important oxidizing functions. The reduction of oxidation can also be shown experimentally by studying the respiratory exchange, Welsch⁵ having found the oxidation

¹ An interesting exception has been reported by W. G. Smith (Practitioner, 1903 (70), 155) who found great quantities of leucin in the urine of a young woman who was apparently not at all ill.

² Berl. klin. Woch., 1905 (42), No. 44 a., p. 54.

³ Salkowski (Berl. klin. Woch., 1905 (42), 1581) found in the urine of a case of acute yellow atrophy a large quantity of nitrogen in a colloidal but non-proteid form, apparently of carbohydrate nature. Mancini (Arch. di farm. speriment., 1906, Bd. v) also observed an increase in the colloidal nitrogen of the urine in liver diseases.

⁴ See Welsch, Arch. int. pharm. et Thér., 1905 (14), 211.

⁵ Loc. cit.

decreased by from $\frac{1}{9}$ to $\frac{1}{5}$ in phosphorus poisoning. Carbamates do not seem to be present in recognizable amounts, and sugar is also absent.

In **phosphorus poisoning** the urinary findings are similar, but with marked quantitative differences. Tyrosin cannot usually be detected, at least by ordinary methods, being found by Riess in but 7 of 36 cases of (human) phosphorus poisoning, and in but 4 of these was it abundant. Leucin is even less frequently found. With experimental animals glycocoll and other amino-acids have been found¹ in the urine, and they could probably be found in acute hepatic atrophy if the same delicate methods were employed. Wohlgemuth² has indeed found glycocoll, alanin, and arginin in human urine after phosphorus poisoning. The small quantity of amino-acids in phosphorus poisoning is probably due to the relative slowness of the autolytic changes. On the other hand, the deficiency of oxidation in phosphorus poisoning is shown by the abundant elimination of organic acids, Riess having obtained as high as 4 to 6 grams of the zinc salt of *paralactic* acid from the urine (per liter) in human cases, and its presence seems to be constant.

The Liver.—In the liver may be found an abundance of the free amino-acids that have not yet escaped by diffusion, their presence having been first detected by Frerichs microscopically. Taylor³ was able to isolate from a liver weighing 900 grams 0.35 gm. of leucin and 0.612 gm. aspartic acid, which probably represent much less than the total amount present. Deuteroalbumose was also found, but no peptone, arginin, histidin, or lysin, and glycogen was also absent. In another case that appeared to be the result of chloroform intoxication, Taylor⁴ obtained 4 grams of leucin, 2.2 grams of tyrosin, and 2.3 grams of arginin nitrate. Wakeman⁵ found that in phosphorus poisoning of dogs the liver shows a diminution of the hexone bases as a whole, the arginin being especially reduced; and Wohlgemuth⁶ found arginin in the urine in phosphorus poisoning. The lecithin of the liver is also decreased (Heffter⁷), and the increase in P_2O_5 observed in the urine presumably comes partly

¹ Abderhalden and Barker, *Zeit. physiol. Chem.*, 1904 (42), 524; Abderhalden and Bergell, *ibid.*, 1903 (39), 464.

² *Zeit. physiol. Chem.*, 1905 (44), 74.

³ *Zeit. physiol. Chem.*, 1902 (34), 580; *Jour. Med. Research*, 1902 (8), 424.

⁴ Univ. of Calif. Publications (Pathol.), 1904 (1), 43.

⁵ *Jour. Exper. Med.*, 1905 (7), 292.

⁶ *Zeit. physiol. Chem.*, 1905 (44), 74.

⁷ *Arch. exp. Path. u. Pharm.*, 1891 (28), 97.

from this source. Beebe¹ found the pentose of the liver not greatly altered from the normal relations. The typical idiopathic atrophied liver shows *little or no increase in fat*, either chemically or microscopically, whereas there is considerable replacement of the lost liver substance by water, as shown in the following table²:

	Water.	Fat.	Fat-free dried substance.
Normal liver	76.1	3.0	20.9
Acute atrophy (Perls)	87.6	8.7	9.7
“ “ (Perls)	76.9	7.6	15.5
“ “ (v. Starck)	80.5	4.2	15.3
Phosphorus-poisoning (v. Starck) . . .	60.0	29.8	10.0
Fatty degeneration (v. Starck)	64.0	25.0	11.0

Similar results have been obtained frequently by other observers, Taylor estimating that in his case about three-fourths of the liver parenchyma had disappeared. The yellow color of the liver tissue characteristic of this condition seems to be due to bilirubin rather than to fat, because as soon as the tissues are put into oxidizing agents (*e. g.*, dichromate hardening fluids) they turn grass-green from the oxidation of the bilirubin into biliverdin.

Jacoby³ found that the livers from phosphorus-poisoned dogs underwent autolysis with greater rapidity than normal livers, which was attributed to increased activity or amount of the autolytic enzymes, although addition of phosphorus to a solution containing liver ferments was not found to increase their activity. The aldehydase was not found decreased, and tyrosinase could not be demonstrated.

The Blood.—In the blood marked changes are found, one of the most prominent, besides the icterus, being the *decreased coagulability* of the blood. This seems due to a loss of fibrinogen, which, with the globulin, is greatly decreased, the albumin remaining less altered.⁴ The fibrin-ferment also seems to be decreased. These changes may be due to direct autolysis of the blood constituents (Jacoby having found that thrombi become rapidly dissolved in phosphorus-poisoning) or to the changes in the liver. The icterus depends apparently upon

¹ Amer. Jour. of Physiol., 1905 (14), 237.

² From Quincke, Nothnagel's System, 1899, vol. 18, p. 297.

³ Zeit. physiol. Chem., 1900 (30), 174.

⁴ Jacoby, *loc. cit.*; see also Doyon, Compt. Rend. Soc. Biol., 1905 (58), 493.

lesions of the finest bile capillaries,¹ although there is also some increase in hemolysis, and a decrease in the total blood and all its elements (Welsch²); and both bile salts and pigments appear in the urine. Neuberg and Richter³ have analyzed the blood drawn during life from a patient with acute hepatic atrophy, and isolated from 355 c.c. of blood 0.787 gm. tyrosin, 1.102 gm. leucin, and 0.240 gm. of lysin; they estimated the amount of free amino-acids in the entire blood to be about 30 grams. This amount is so large that they question the possibility of it all arising from the degenerated liver tissue; but more analyses are necessary before conclusions on this point can be drawn.⁴

Origin of the Amino-acids.—The earliest conception of the source of the leucin and tyrosin found in the urine was that it came from the products of tryptic digestion absorbed from the intestinal tract, which the liver could not convert into urea because of its damaged condition. On the demonstration by Jacoby⁵ that these same bodies were present in the livers of phosphorus-poisoned animals because of autolysis, it became probable that the leucin and tyrosin found in the urine were formed from the degenerated liver-cells rather than in the intestine, which view has become generally accepted. In support of this view are also the observations which indicate that the amino-acids formed in the intestine are resynthesized or otherwise altered in passing through the intestinal wall. Neuberg and Richter have, however, suggested that the urinary amino-acids are, at least in part, derived from the intestinal contents, assuming that they may pass unaltered through the intestinal wall because of pathological alterations in its structure. It seems most probable that the urinary amino-acids are derived partly (and perhaps chiefly) from the autolysis of the liver, and partly from amino-acids produced both in the intestine and within the body during tissue metabolism, and which the liver cannot transform into urea as it normally does.

¹ Lang (Zeit. exp. Path., 1906, Bd. 3, July) found fibrinogen in the bile of a dog poisoned with phosphorus, which may account for the occlusion of the bile vessels and the resulting jaundice.

² Arch. int. Pharm. et Thér., 1905 (14), 197.

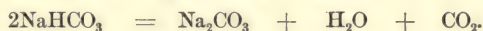
³ Deut. med. Woch., 1904 (30), 499.

⁴ v. Bergmann (Hofmeister's Beit., 1904 (6), 40) was able to isolate 2.3 grams of amino-acids combined with the chloride of naphthalene sulphonie acid, from 270 c.c. of blood in a case of acute yellow atrophy.

⁵ Zeit. physiol. Chem., 1900 (30), 174.

ACID INTOXICATION¹

If a rabbit is given in repeated small doses by mouth considerable quantities of inorganic acids, such as hydrochloric or phosphoric acids, which it cannot destroy by oxidation, it soon becomes extremely sick. The manifestations are characteristic—unsteadiness of motion and stupor being followed by coma, in which the striking feature is the excessively active respiration, as if the animal were being asphyxiated (the so-called “air hunger”), while at the same time there is no cyanosis and the blood is bright red, containing much less CO_2 than normal, while the amount of oxygen remains quite normal. The explanation of this interesting condition is as follows: Normally the blood carries the CO_2 away from the tissues to the lungs in combination with the inorganic alkalies of the blood, of which sodium is by far the most abundant. This combination is the bicarbonate of sodium (or other base), which in the lungs is decomposed into the carbonate, the CO_2 escaping into the alveolar air, according to this equation:



The carbonate thus formed goes back to the tissues to again combine with more CO_2 and form bicarbonate. If acids are introduced into the blood they combine with the alkalies there, forming neutral salts which are eliminated in the urine, and in this way the amount of alkali in the blood is reduced, with a consequent reduction in the capacity of the blood to carry CO_2 away from the tissues; the amount of CO_2 in the blood sinking from the normal 24 per cent. to as low as 2.5 and 3 per cent. (Walter). Consequently, in acid poisoning the CO_2 produced in metabolism accumulates in the tissues where it is formed, and blocks the processes of oxidation, so that the animal suffers from asphyxia exactly as if it were deprived of air. In other words, the lack of alkalies in the blood in acid intoxication checks the “internal respiration,” as intracellular gas exchange is called, by preventing the removal of CO_2 from the cells.

If the urine of such an animal is analyzed, it is found to contain increased quantities of the four chief inorganic bases, Na, K, Ca, and Mg (the last two apparently being derived from the bones), but in addition to these it is found that the amount of ammonia in the urine is decidedly increased. If instead of

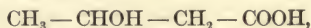
¹General literature will be found in Waldvogel's “Die Acetonkörper,” Stuttgart, 1903; v. Noorden's “Die Zuckerkrankheit und ihre Behandlung”; in Krehl's “Pathologische Physiol.,” pp. 397–406; and in the articles cited in the text.

a rabbit a carnivorous animal, such as a dog, is given acids, it will be found relatively insusceptible, so that great quantities can be given without causing acid intoxication. Examination of the urine of such a dog will show that the elimination of ammonia is increased much more than it is in the herbivora, while the inorganic alkalies are relatively increased but little. From this it is deduced that in acid intoxication part of the nitrogen that normally goes to form urea becomes, while in the antecedent form of ammonia, combined with part of the acid that has entered the blood. In this way much of the neutralization of the acids is accomplished by ammonia, and the inorganic alkalies of the blood are spared. As in carnivora the amount of proteid metabolism is much greater and more rapid than in herbivora, the ammonia available for neutralization of acids is much greater than in the latter, and hence the relative lack of susceptibility of carnivora to acid poisoning.¹ According to Landau,² the proteids of the blood also combine much of the acid—probably one-half of it and perhaps more.

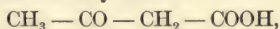
DIABETIC COMA³

In man, poisoning with inorganic poisons, as in the experiments cited above, is a rare occurrence, but not infrequently acid intoxication results from the presence of undue quantities of organic acids produced in metabolism. The most striking example of this is the coma of diabetes, in which the asphyxia without cyanosis, dependent upon failure of the blood to carry CO₂, is strikingly similar to that observed in experimental animals poisoned with acids. In diabetic coma the acid intoxication is due chiefly to the accumulation in the blood of large quantities of *β-oxybutyric acid*. Associated with it, in smaller quantities, are usually found *diacetic (aceto-acetic) acid* and *acetone*, which are chemically so closely related that it is generally considered that they are derived from the oxybutyric acid, as follows:

β-oxybutyric acid is—



and by oxidation this readily forms—

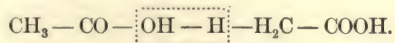


¹This has been nicely shown by Eppinger (Wien. klin. Woch., 1906 (19), 111), who found that administration of considerable quantities of amino-acids (glycocoll, alanin, aspartic acid) to rabbits greatly increased their resistance to acid intoxication, presumably by yielding ammonia through the normal steps of proteid metabolism.

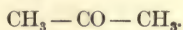
²Arch. exp. Path. u. Pharm., 1900 (52), 271.

³See also v. Noorden's "Diabetes Mellitus," 1905, New York.

which is diacetic acid (being two molecules of acetic acid united to each other, as follows) :



Diacetic acid is, in turn, readily deprived of its COOH group through oxidation, forming acetone,



All these reactions are readily accomplished in the laboratory, and we have good reason for believing that they normally occur in the same way in the animal body. (Because of their chemical relation these substances are often referred to collectively as the "*acetone bodies*"). As long as a diabetic is maintaining a good metabolic equilibrium the urine is free from both acids, although small amounts of acetone (traces of which—under 0.02 gm. per day—occur in normal urine¹) may be present; but when wasting sets in the two acids appear, combined largely with ammonia, but partly with mineral bases. Normally but 2 to 5 per cent. of the nitrogen of the urine is in the form of ammonia, but in diabetic *acidosis* the proportion may reach from 10 to 25 per cent., the amount of urea being correspondingly reduced.²

The presence of large quantities of these acids in the urine presages coma, during which the amount of oxybutyric acid often reaches 15–20 grams per day, and has been known to reach 150 grams (Külz claimed to have found 226 grams). Diacetic acid appears in relatively small amounts, rarely exceeding 10 per cent. of the total organic acids of the urine. When oxybutyric acid is present the other two substances are always present,³ but the converse is not true. In the development of acetonuria, acetone is the first of the three bodies to appear; when 0.4 to 0.5 gm. of acetone is present in the day's urine diacetic acid may be found, but oxybutyric acid does not usually appear until the amount of acetone exceeds 1 gram. After this the chief increase is in the oxybutyric acid, which often reaches 30 to 80 grams, whereas the diacetic acid and acetone together rarely exceed 7 to 8 grams (v. Noorden). In the internal organs the acetone bodies may also be detected. Geelmuyden⁴ found that the organs of diabetics contain consider-

¹ Concerning normal occurrence of acetone in blood and tissues, see Halpern and Landau, *Zeit. exp. Path. u. Ther.*, July, 1906, Bd. 3.

² According to Edie and Whitley (*Biochemical Jour.*, 1906 (1), 11), administration of excessive amounts of alkali causes, conversely, elimination of increased amounts of organic acids.

³ See Pavy, *Lancet*, 1902 (ii), 64 *et seq.* (general review).

⁴ *Zeit. physiol. Chem.*, 1904 (41), 128.

able acetone, the liver less than the other viscera; the blood contains less acetone than the urine of the same patient.

Relation of Acidosis to Diabetic Coma.—There seems to be little room for doubt but that the typical diabetic coma with “air hunger” depends upon an excess of these substances in the blood—*i. e.*, is an acid intoxication—for the following reasons: (1) The coma appears when the amount of organic acids in the urine is highest, and is absent when there is little or none of them in the urine. (2) Because of the identity of the symptoms with those of experimental acid intoxication. (3) Because of the repeated demonstration of a reduced amount of alkali in the blood, as determined by titration,¹ and a great reduction of the amount of CO₂ carried in the venous blood (from the normal 36 per cent. it may be reduced to 3.3 per cent.—Minkowski). (4) The marked improvement that often results from the administration of alkalies (usually sodium bicarbonate). Associated with this improvement is an elimination of greatly increased amounts of organic acids, indicating their previous retention in the body because of lack of alkali with which they could combine (or their liberation from combination with proteids—Landau).

β -oxybutyric and diacetic acid, according to many authorities, seem to have no specific poisonous effects,² but act simply as acids in the blood.³ Acetone does not have this effect, not being an acid, and seems not to be toxic to any considerable degree; doses of 4 grams per kilo cause effects similar to ethyl alcohol in dogs, 8 grams per kilo being fatal, which corresponds with a dose of 500 grams for an adult man. Of course a diabetic suffers from the effects of other poisons than these acids, and often the coma cannot be relieved by alkaline treatment, and seems not to be due to the acids alone. But, in the majority of cases, the acids seem to be the chief factor, as shown by the marked effect of alkaline treatment.

¹ The actual alkalinity of normal blood, which means the number of free OH ions, is but little greater than that of distilled water, and the condition is quite the same in diabetic acidosis (Benedikt and Török, cit. in *Folia Hematologia*, 1905 (2), 454).

² Some, however, attribute to oxybutyric acid a considerable toxicity independent of its acidity.

³ The view advanced by Sternberg (*Zeit. f. klin. Med.*, 1899 (38), 65) that an antecedent of oxybutyric acid, namely, *amino-butyric acid*, is responsible for the intoxication, does not seem to have been generally accepted, although Grube (*Arch. f. exp. Pathol.*, 1900 (44), 349) found that α -amino-butyric acid is toxic and produces symptoms similar to those of diabetic coma. Magnus-Levy questions the possibility of sufficient amino-butyric acid being present to account for the great amount of acid eliminated in the urine (*Arch. exp. Path. u. Pharm.*, 1901 (45), 389).

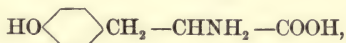
Origin of the Acetone Bodies.—As yet we are uncertain as to the origin of these acetone bodies. Their close chemical relation with one another makes it seem probable that they have a common source, and it is also probable that they are not abnormal products of metabolism, produced only in patients with acid intoxication, but that they are formed normally in metabolism, and accumulate when they cannot be destroyed as they normally are, through oxidation. Acid intoxication, therefore, is dependent upon a failure of complete oxidation of organic acids produced in metabolism. But whether the acids are formed from fats, or from carbohydrates, or from proteids, or from all three, has not been conclusively determined. Their chemical nature is such that they might readily be produced from any or all of the three classes of food-stuffs.

They might be derived from *carbohydrates*, as is the closely related lactic acid, but it is generally believed that this is not the usual source, particularly because administration of a proper amount of carbohydrates under certain conditions may cause the acids to disappear from the urine,¹ and because the acids may be eliminated in large quantities while the patient is on a diet almost free from carbohydrates.

They might readily be formed from *proteids* through splitting out of the NH_2 group from the amino-acids, just as in acute yellow atrophy we may find in the urine oxymandelic acid,



derived from tyrosin,



by removal of the nitrogen-containing radical, with subsequent failure of normal oxidation of the non-nitrogenous residue. Indeed the amino-acids are generally considered as the chief source of the acetone bodies,² particularly because whenever there is considerable pathological breaking-down of proteids these bodies, especially acetone, may appear in the urine; *e. g.*, in patients with retained placenta or dead fetus, during absorption

¹ See Satta, Hofmeister's Beitr., 1905 (6), 376.

² Embden and his associates have recently (Hofmeister's Beitr., 1906 (8), 121) demonstrated that the liver can form acetone from many substances perfused through it in the blood, including not only amino-acids of the fatty acid series, but also from the aromatic radicals of the proteid molecule.

of exudates, in carcinoma, and in starvation or other conditions with great wasting of the tissues.¹

On the other hand, the amount of acids sometimes found in the urine seems to be greater than can be explained by the proteid destruction that occurs (Magnus-Levy²), and hence it has been thought that acetone bodies may be derived from the *fats*. β -oxybutyric acid can be readily produced from fatty acids, especially, of course, from butyric acid, and Schwarz observed an increase in the acetone excretion in a diabetic given large quantities of butter. Other higher fatty acids were also found to cause increased acetone excretion. Joslin,³ however, found that butyric acid does not increase the acetonuria of a healthy, fasting individual, nor do neutral fats; oleic acid and sodium palmitate, on the other hand, caused a marked acetonuria. It is furthermore possible, but this is purely hypothetical, that the acetone bodies may be synthesized from other simpler substances.

Sarcolactic acid appears in the urine, particularly when the liver is badly incapacitated, and, therefore, is rarely found in diabetes, but is a prominent finding in phosphorus-poisoning, acute yellow atrophy, and in puerperal eclampsia. (See preceding sections of this chapter.) The amount is never sufficient to cause an acid intoxication by abstraction of alkali from the blood, nor does it seem to possess sufficient toxicity to cause all of the manifestations of puerperal eclampsia, as has been suggested. It is normally present in the muscles, being produced in increased amounts during exercise, and therefore it may appear in the urine after violent and protracted muscular exertion; apparently this acid is destroyed in the liver through oxidation, and therefore appears in the urine when the liver is disorganized, but there is also much reason for believing that under these conditions the sarcolactic acid found in the urine comes from the disintegrating cells themselves.⁴ Sarcolactic acid, which is dextrorotary, must be distinguished from its optical isomer, the inactive lactic acid that is produced by fermentation. When this fermentation lactic acid is formed in

¹ Résumé by Mauban, Thèse de Paris, 1905.

² Arch. exp. Path. u. Pharm., 1899 (42), 149.

³ Jour. Med. Research, 1904 (12), 433.

⁴ Mandel, however, considers that sarcolactic acid comes from carbohydrates, since phosphorus-poisoning does not cause the appearance of lactic acid in the urine of dogs with experimental (phlorhizin) diabetes, and when produced by phosphorus-poisoning the administration of phlorhizin checks it (Amer. Jour. Physiol., 1905 (13), p. xvi); also see Mandel and Lusk, *ibid.*, 1906 (16), 129.

the stomach and enters the blood, it ordinarily, like other ingested organic acids, is combined by the blood alkalies and oxidized to carbonates. It is doubtful if it ever enters the urine.¹

ACID INTOXICATION IN CONDITIONS OTHER THAN DIABETES

Not infrequently acetone and diacetic acid, less often oxybutyric acid, are found in the urine of patients suffering from the most diverse diseases. It is customary to refer to this condition as "*acetonemia*" or "*acetonuria*," and to ascribe many of the observed symptoms to "acid intoxication." The acetone bodies, however, being without specific toxic effects, can probably cause only such symptoms as described in discussing diabetic coma, and these are due to their reducing the carrying power of the blood for CO_2 . Therefore, the intoxication in these cases is probably not due to the acids, but, on the contrary, the presence of the acetone bodies is due more often to the effects upon the liver of toxic substances of diverse origins and natures. In no other condition do the amounts of organic acids in the urine approximate the amounts found in diabetic coma.

Anesthesia.—Most prominent of these so-called acid intoxications is that following a few days after anesthesia, particularly with chloroform, and fully discussed by Bevan and Favill.² As shown first by Greven (1895), and more recently especially by Brewer and by Helen Baldwin,³ acetone is nearly always present in the urine during the first twenty-four hours after administration of either chloroform or ether, and occasionally diacetic acid appears on the second or third day after; but ordinarily there is no increase in organic acids in the urine. It does not seem probable that the symptoms observed in typical cases of delayed chloroform-poisoning are due chiefly, if at all, to acid intoxication *per se*, but rather are the result of extensive injury to the parenchymatous organs, particularly the liver, by the chloroform, which causes a condition resembling acute yellow atrophy or phosphorus-poisoning.⁴

Cachectic Acetonuria.—Acetone and diacetic acid, but less abundantly the oxybutyric acid, are found in the urine in many conditions associated with *wasting*, among which may be especially mentioned:

¹ The theory of Boix that cirrhosis of the liver may be produced by butyric acid formed in gastric fermentation could not be corroborated by Joannovics, Arch. int. Pharmacodyn., 1905 (15), 241.

² Journal Amer. Med. Assoc., 1905 (45), 691.

³ Jour. of Biol. Chem., 1906 (1), 239.

⁴ Wells, Jour. Amer. Med. Assoc., 1906 (46), 341.

Infantile marasmus,¹ in which increased ammonia is found in the urine, and sometimes symptoms resembling acid intoxications occur. Normally the urine of suckling infants contains 1–4 mg. per day of acetone bodies, which may be increased to 15–35 mg. by simple hunger. In fact, “acidosis” seems to occur particularly frequently in infants from relatively slight causes, such as gastro-enteritis and other infectious conditions. This is perhaps due to a lower oxidizing power on the part of the infantile organism (Pfaundler²), since the proportion of nitrogen in the urine of infants in forms other than urea, is higher than in adults. Even an unusually fatty diet may cause acetonuria in infants.

Cancerous cachexia, in which a “cancer coma” occasionally occurs that is strikingly like that of diabetic coma.

Retention of placenta or fetus, acetonuria being considered of diagnostic value in determining the death of the fetus *in utero*.³

Pernicious vomiting of pregnancy is often associated with acetonuria,⁴ which in some cases is probably dependent upon starvation and proteid wasting,⁵ but in other cases is probably due to liver alterations resembling those of puerperal eclampsia or acute yellow atrophy.⁶ Williams⁷ considers that this condition may result from three varieties of etiological factors, namely—reflex, neurotic, and toxemic. Only in the last form, which is associated with marked degenerative changes in the liver, are there striking metabolic changes. These are indicated by a marked increase in the ammonia nitrogen of the urine, which he has observed to form as much as 46 per cent. of the total nitrogen. Starvation seldom causes a rise in the ammonia quotient above 10–15 per cent., and Williams considers that an ammonia quotient of over 16 per cent. is an indication for the interruption of pregnancy, and even then the prognosis is dubious.

In **febrile diseases**, especially in children, acetonuria is often observed, apparently depending on wasting of the tissue proteids.

In **uremia**, as previously mentioned, organic acids may appear in the urine, but apparently as a result, and not as the cause, of the uremia (Orlowski).

¹ See Meyer and Langstein, *Jahrb. f. Kinderheilk.*, 1906 (63), 30.

² *Jahrb. f. Kinderheilk.*, 1901 (54), 247.

³ See Frommer, *Berl. klin. Woch.*, 1905 (42), 1008.

⁴ Baldwin, *Amer. Jour. Med. Sci.*, 1905 (130), 649.

⁵ Wolf, *New York Med. Jour.*, 1906 (83), 813.

⁶ Ewing, *Amer. Jour. of Obstet.*, 1905 (51), 145.

⁷ *Johns Hopkins Hosp. Bull.*, 1906 (17), 71; full bibliography.

Mauban¹ distinguishes the following groups of conditions causing acetonuria: Physiological acetonuria, diabetes, febrile diseases, carcinoma, resorption of tissues and exudates, gastroenteritis, nervous diseases, general anesthesia, and inanition.

As mentioned in discussing these diseases, lactic acid has been found in the urine in *osteomalacia* and in *rickets*, but the attempts to explain these diseases as due to solution of the bone salts by the organic acids have not met with success. (See "Calcification," Chap. xv). In *rheumatism* lactic acid is said to have been found in the urine and sweat, but these results have not been verified, particularly as to the sweat, and the once prominent idea that rheumatism is due to an acid intoxication seems to have been given up.² In *rheumatoid arthritis*, as shown by Herter and by Baldwin,³ there is an excessive elimination of organic acids of undetermined nature in the urine.

FATIGUE

The symptoms of fatigue, whether general or local, seem to be due to an intoxication with the products of the excessive metabolic activity, and part of the symptoms, at least, seem to be due to acid intoxication. Among the metabolic products of muscular activity are known to be creatin, creatinin, sarcolactic acid, and carbon dioxide. The amount of acid developed in an active muscle is quite considerable, and when the activity is violent or prolonged the sarcolactic acid accumulates, being formed faster than it can be removed. Part of the acidity of the muscle is due, however, not to the sarcolactic acid itself, but to monopotassium phosphate (KH_2PO_4), which is formed by the action of the sarcolactic acid upon the dipotassium phosphate present in the blood and muscle. The effect of these various substances upon muscular fatigue has been studied experimentally, and while the creatin seems not to be a "fatigue substance," sarcolactic acid, monopotassium phosphate, potassium sarcolactate, and carbon dioxide all cause muscle tissue to react to stimuli in the same way that a fatigued muscle does (Lee⁴).

It is quite probable that the muscular weakness of diabetics, and the exhaustion associated with many conditions in which organic acids appear in the urine in abnormal quantities, depend,

¹ Thèse de Paris, 1905.

² See Garrod, *Treatise on Rheumatism*, 1890. Walker and Ryffel (Brit. Med. Jour., 1903 (ii), 659, report finding *formic acid* in the urine in acute rheumatism.

³ Amer. Jour. Med. Sci., 1904 (128), 1038.

⁴ Jour. Amer. Med. Assoc., 1906 (46), 1491; where is given a complete review of the subject of fatigue, with the literature.

at least in part, upon the effect of these acids upon the muscle tissue, for Lee found that β -oxybutyric acid causes the same fatigue reaction in muscles as does sarcolactic acid. Furthermore, sarcolactic acid itself often appears in the urine in these conditions. It may be added that in fatigued animals the alkalinity of the blood (by titration) has been found decreased (Geppert and Zuntz), and the proportion of the urinary nitrogen that appears in other combinations than urea is increased (Poehl¹).

The "Toxins" of Fatigue.—In extreme exhaustion the evidences of a general intoxication often become severe, so that the condition may resemble an acute febrile disease and last for several days. It seems very probable that substances more toxic than the above-mentioned acids are involved. Weichardt² claims to have demonstrated as produced by muscular fatigue a toxic substance, which in structure resembles the bacterial toxins, and against which an antitoxin may be obtained. This toxic material is, he believes, formed from the proteid molecule in the first stages of its decomposition, as a side product which is normally protected against by a formation of an antitoxin, rather than by being split up further, as is the case with the rest of the proteid molecule.³ Whether this work is confirmed or not, there remains the fact that the blood of fatigued animals contains toxic substances, which Mosso proved as follows: If blood is transfused from an exhausted dog to a normal dog, from which an equivalent amount of blood has been withdrawn, this second dog will show the usual manifestations of fatigue.

Mental Fatigue.—The chemical changes of mental fatigue are not known, but it is known that the ganglion-cells show marked structural alterations as a result of fatigue, chromatolysis often being very striking. Since lecithin forms so important a part of the nervous system, it is tempting to imagine that in fatigue excessive quantities of its toxic decomposition-product, *cholin*, and the still more toxic derivative of cholin, *neurin*, are formed in considerable amounts and cause part, at least, of the intoxication. Cholin has been demonstrated by Halliburton and Mott and their co-workers, in the cerebrospinal fluid, and also sometimes in the blood of patients suffering from organic nervous lesions, including such conditions as disseminated sclerosis, tabes dorsalis, progressive muscular atrophy,

¹ Dent. med. Woch., 1901 (27), 796.

² Münch. med. Woch., 1904 (51), 12 and 2121; 1905 (52), 1234; also reviewed by Wolff-Eisner, Cent. f. Bakt., 1906 (40), 634.

³ Weichardt, Münch. med. Woch. 1906 (53), 7.

transverse myelitis, and especially in general paresis.¹ That it or neurin actually is the cause of any of the symptoms of fatigue, however, has not been established; but Donath² considers cholin an important factor in the production of *epileptic convulsions*. (Concerning the theories and literature of the subject of epilepsy in relation to its pathological chemistry and to autointoxication, see the review by Masoin.³)

THE POISONS PRODUCED IN SUPERFICIAL BURNS⁴

In a certain proportion of cases of extensive but superficial burns, death follows after an interval of from six hours to a few days, apparently because of a profound intoxication. As evidence of intoxication we have not only clinical manifestations, such as delirium, hemoglobinuria, and albuminuria, vomiting, bloody diarrhea, etc., but, more convincingly, the anatomical findings at autopsy, which are strikingly similar to those resulting from acute intoxication with bacterial products. Bardeen found quite constantly cloudy swelling and focal and parenchymatous degeneration in the liver and kidneys; softening and enlargement of the spleen with focal degeneration in the Malpighian bodies; and particularly degenerative changes in the lymph-glands and intestinal follicles resembling those observed in diphtheria, which McCrae⁵ considers due to proliferation and phagocytosis by the endothelial cells of the lymphatic structures. Marked changes are usually present in the blood, consisting of fragmentation and distortion of the red corpuscles, hemoglobinemia, loss of water with a relative increase in the number of corpuscles by from one to four millions per cubic millimeter, an increase in the blood platelets, and a rise in the number of leucocytes as high as 30,000 to 50,000.⁶ Hemoglobinuria is also frequently present, and almost constantly gastro-intestinal irritation occurs, with anatomical evidences of acute enteritis, acute gastritis, and occasionally gastric or duodenal ulcers. According to Korolenko,⁷ the sympathetic nervous system is seriously involved.

¹ Halliburton, "Biochemistry of Muscle and Nerve," 1904, p. 116; Donath, *Jour. of Physiol.*, 1905 (33), 211.

² *Zeit. physiol. Chem.*, 1903 (39), 526.

³ *Arch. internat. de Pharmacodynamie*, 1904 (13), 387.

⁴ Literature given by Bardeen, *Johns Hopkins Hosp. Reports*, 1898 (7), 137; Eyff, *Cent. Grenzgeb. Med. u. Chir.*, 1901 (4), 428; Pfeiffer, *Virchow's Arch.*, 1905 (180), 367.

⁵ *Amer. Med.*, 1901 (2), 735.

⁶ Locke, *Boston Med. and Surg. Jour.*, 1902 (147), 480.

⁷ *Cent. f. Path.*, 1903 (10), 663.

It therefore seems probable that poisons are formed as a result of superficial burns, which have the effect of causing hemolysis, and which are also cytotoxic for parenchymatous cells and particularly for nervous tissues. These hypothetical poisons seem to be eliminated by the intestines and kidneys, which are injured by the poisons in their passage through these organs. The attempts to explain all the observed effects of burns as due to thrombosis or to embolism by altered red corpuscles seem to have failed, for the peculiar location of the lesions (*e. g.*, duodenal ulcers, necrosis in the Malpighian bodies of the spleen, etc.) does not agree with this hypothesis, and there are too many evidences of the presence of some decidedly toxic substance in the blood. There can be no question that the poisonous substance or substances are formed in the burned area, and not in the internal organs as a result of hyperpyrexia, as shown by numerous observations. Thus, if the burned area is removed immediately (in narcotized experimental animals), death will be prevented, whereas if the burned tissue is permitted to remain for a few hours, death will occur. The poison appears to be absorbed from the burned area into the blood, for if the circulation is shut off from the burned area, no intoxication results; this probably explains in part why deep destructive burns of small areas, which are associated with local thrombosis, are much less serious than a superficial slight scalding over a large area. Apparently the poison is produced chiefly or solely in the skin, for burning of muscle is not followed by intoxication (Eijkman and Hoogenhuyze¹). Numerous investigators have reported finding poisonous substances in the blood, tissues, or urine of burned men and animals, but the reports disagree widely in details.² Thus Dietrichs states that the blood of burned animals contains hemolysins and hemagglutinins, which could not be corroborated by Burkhardt³ or by Pfeiffer.⁴ The latter, however, finds that the urine, serum, and organs of burned animals contain substances poisonous for the same and for different species, which is in accord with the results of numerous earlier investigators. The poisons, according to Pfeiffer are neurotoxic and necro-

¹ Virchow's Arch., 1906 (183), 377.

² Ravenna and Minassian (ref. in Biochem. Centr., 1903 (1), 348) state that blood heated outside the body to 55°-60° is toxic, and causes the same anatomical changes as does death from burning, which finding is corroborated by Helsted (Dissertation, Copenhagen, 1905; abst. in Nordiskt Med. Ark., 1906 (39), July 11).

³ Arch. klin. Chir., 1905 (75), 845.

⁴ Virchow's Arch., 1905 (180), 367.

genic in their properties, and act without a period of incubation; they are rapidly weakened on standing in solution and by the action of sunlight, are absorbed from the gastro-intestinal tract, are soluble in water, alcohol, and glycerin, but not in chloroform or ether, are precipitated by HgCl_2 in acid solution, and by phosphotungstic acid, and they are not volatile. Apparently, according to Pfeiffer, they are not ptomaines, nor yet pyridin derivatives, as many investigators have contended, but resemble more closely the labile poisons of snake venom. The neurotoxic substance is more thermostable than the necrogenic substance, which is very easily destroyed by heat. Pfeiffer believes it probable that the poisons are derived from the cleavage of proteids altered in composition by burning. The hemolysis he attributes to direct injury of the blood in its passage through the heated area, and not to the action of poisons; this is very possible, since red corpuscles fragment after being heated to 52° , and may be seriously impaired functionally at 45° . There are many authors, indeed, who consider the blood changes the chief cause of death, but the weight of evidence is in favor of the theory of the development of toxic substances in the burned skin. In spite of Pfeiffer's researches, however, the nature of these poisons must be considered as completely unknown, for numerous other observers have described "peptotoxins" (Fraenkel and Spiegler), ptomaines (Kijanitzin, Ajello and Parascendolo), and pyridin bases (Fraenkel and Spiegler, Reiss¹). It remains also to be determined if the poisons are of such a nature that an immune serum can be obtained for them.

Burn Blisters.—The contents of burn blisters resemble the fluid of inflammatory edemas generally. K. Mörner² found 5.031 per cent. of proteids, which included 1.359 per cent. of globulin and 0.011 per cent. of fibrin; there was also present a substance reducing copper oxide, but no pyrocatechin.

¹ References given by Pfeiffer, *loc. cit.*

² Skand. Arch. Physiol., 1895 (5), 272.

CHAPTER XIX

GASTRO-INTESTINAL "AUTOINTOXICATION" AND RELATED METABOLIC DISTURBANCES

UNDER this heading are commonly included all intoxications that can be ascribed to the absorption from the gastro-intestinal tract of toxic substances that have been formed within its contents, either by the action of the digestive ferments or of putrefactive bacteria. The propriety of considering such conditions as examples of autointoxication is properly questioned, since it is often difficult to determine whether the putrefaction occurred within the body, or had already taken place in the food before it was eaten. But even those who would limit the use of the term autointoxication to intoxication with the products of cellular metabolism, must admit the possibility of products of metabolism reëntering the blood from the contents of the bowels through the intestinal wall, since the bile, and perhaps also the intestinal juice, contain excrementitious substances which may, in case of defective fecal elimination, be reabsorbed into the blood. Therefore, in gastro-intestinal disturbances we have the possibility of both true autointoxication and intoxication by putrefactive products occurring together in an inseparable way, and the common inclusion of gastro-intestinal intoxication in the discussion of autointoxication would seem to be justifiable as well as expedient.

The sources of poisonous substances arising in the gastro-intestinal tract are numerous. They may be formed either from the food-stuffs, or from the secretions and excretions of the body that enter the alimentary canal; and they may be formed either by the digestive ferments or by the bacteria of the intestinal contents. Hence the number of these products is enormous, and we are by no means sure that those that have yet been identified include the most important or most toxic. To classify the poisonous substances that are known to be formed in the alimentary canal, and which might, under certain conditions, cause an intoxication, is extremely difficult, because of the uncertainty of our information; but, using as a basis the sources of the substances, they may be classified as follows:¹

¹ Modified from Weintraud, *Ergeb. allg. Pathol.*, 1897 (4), 1, who gives exhaustive discussion and bibliography to that date.

I. The constituents of the digestive secretions, including the bile salts and pigments, pepsin, and trypsin.

II. Products of normal digestion :

(a) From proteids—proteoses, peptones, amino-acids.

(b) From fats—fatty acids and glycerin.

III. Products of putrefaction and fermentation :

(a) From proteids :

(1) From the aromatic radicals (tyrosin, phenylalanin, tryptophan)—indol, skatol, skatol carbonic (or indol acetic) acid, phenol, cresol, dioxyphenols.

(2) From the fatty acid radicals—fatty acids (especially butyric and acetic), acetone, ammonia, amino-acids, carbon dioxide, hydrogen, marsh-gas. Also ptomaines : cadaverin, putrescin, ethylidendiamin.

(3) From the sulphur-containing radicals— H_2S , methyl mercaptan, ethyl mercaptan, ethyl sulphid.

(b) From carbohydrates :

Fatty acids, the following having been detected—formic, acetic, propionic, butyric, valerianic, lactic, oxybutyric, and succinic; also acetone, CO_2 , CH_4 , H_2 .

(c) From fats :

Higher fatty acids, as well as butyric acid; also glycerin. From lecithin—cholin, neurin, and muscarin-like bodies.

IV. Synthetic products of bacterial activity (*e. g.* botulismus) which cannot properly be considered as causing "autointoxication."

I. THE CONSTITUENTS OF THE DIGESTIVE FLUIDS

These call for but brief consideration, for, although many of them are known to be toxic, yet there is no evidence that they cause autointoxication, either in health or disease. Both pepsin and trypsin, especially the latter, are decidedly toxic when injected experimentally into the blood (see *Enzymes*, pp. 77–75), but they do not appear ever to pass through the intestinal wall in sufficient quantity to cause harm, although minute traces may appear in the urine; this harmlessness probably depends largely on the known inhibiting action of the blood upon enzymes.

The bile salts are also toxic, especially hemolytic, but those that are reabsorbed from the intestines are taken back into the liver and reëxcreted. This protective arrangement seems to be sufficient for all emergencies. The bile-pigments become converted into *hydropilirubin* through reduction, and this is largely

absorbed and eliminated as *urobilin*. Icterus and cholemia do not seem ever to be produced by absorption of bile-pigments and bile salts from the intestines. (See Icterus, pp. 405-410.)

II. PRODUCTS OF NORMAL DIGESTION

Proteoses and Peptones.—Under normal conditions, these are broken up in the intestinal wall into the amino-acids, through the agency of erepsin, and do not appear in the blood in appreciable quantities. To be sure, certain authors claim to have found *albumose* in normal blood,¹ but if present the amounts are extremely minute. In conditions in which ulceration or other lesions are present in the gastro-intestinal tract it is possible to find small amounts of proteoses in the urine, probably absorbed through the abnormal areas, but not in quantities sufficient to account for any appreciable intoxication, although proteoses are distinctly toxic. This last statement has been much contested, because the difficulty of purifying proteoses obtained from ordinary sources has left open the possibility that such toxic effects as have been observed are due to contaminating substances, and not to the proteoses themselves. More recent work, however, particularly that of Underhill,² seems to have established affirmatively the toxicity of proteoses, whether from animal or vegetable proteids. Besides the classical effect of inhibiting the coagulation of the blood, the proteoses have a lymphagogue effect (Heidenhain³), cause a fall in arterial pressure, cause a marked febrile reaction, and in doses of some size are fatal to experimental animals (rabbits being much less susceptible than dogs and many other animals⁴). Locally they cause a mild inflammatory reaction, which is followed by the appearance of much connective-tissue formation.⁵

¹ Embden and Knoop, Hofmeister's Beitr., 1902 (3), 120; Langstein, *ibid.*, p. 373; Morawitz and Dietschy, Arch. exp. Path., 1905 (54), 88. However, many investigators have failed to find them; see Abderhalden and Oppenheimer, Zeit. physiol. Chem., 1904 (42), 155; Schryver, Biochemical Journal, 1906 (1), 137; Kraus, Zeit. exp. Pathol., 1906 (3), 52.

² Amer. Jour. Physiol., 1903 (9), 345 (literature).

³ See also Nolf, Arch. internat. de Physiol., 1906 (3), 343.

⁴ According to Buchner and Geret, Münch. med. Woch., 1901 (48), 1163, 0.2 gram of "pure peptone" per kilo kills rabbits in twelve hours.

⁵ In a paper appearing in the Transactions of the Chicago Pathological Society, 1903 (5), 240, I published the observation that repeated injections of Witte's "peptone" (which consists chiefly of proteoses) into rabbits led to the production of marked cirrhosis of the liver, and suggested the possibility that proteoses escaping through a diseased gastric or intestinal wall into the blood might be a factor in the production of cirrhosis in man. Subsequent observations, however, have shown that repeated injection of almost any foreign proteid material (*e. g.*, emulsions of organs, foreign blood, etc., used in immunization experiments) will cause a similar cirrhosis in rabbits, which animals,

According to most observers, *precipitins* for proteoses and peptones cannot be obtained by experimental immunization, although the animal may show a distinct increase in resistance; possibly this failure is not due to a lack of formation of antibodies, but to their forming a compound with proteoses (or peptones) which is soluble, and hence no precipitation results.¹

As the decomposition of the proteid molecule continues, the toxic effects of the resulting substances seem to decrease along with the decreased size of the molecules. Thus Wolf² found that the amino-acids do not cause a fall of blood pressure, nor do polypeptids.³

"Albumosuria."—If proteoses enter the blood stream they appear in large part in the urine, indicating that the tissues do not readily utilize them in this form.⁴ Consequently, when proteoses are produced in considerable amounts by autolysis of pathological tissues they appear in the urine, and their presence is considered to be of diagnostic value.⁵ True peptone seems rarely, and according to many observers never, to appear in the urine. (In view of the recent observations that polypeptids often appear in the urine, it is probable that true peptones also do.) Albumoses, therefore, may be found in the urine whenever any considerable amount of tissue or exudate is being autolyzed and absorbed, and it has been found in the following conditions: Suppuration of all kinds; resolution of pneumonia; involution of the puerperal uterus; carcinoma (two-thirds of all cases—Ury and Lilienthal), and other malignant growths; febrile conditions with tissue destruction (37.5 per cent. of all cases, Morawitz and Dietschy)⁶; acute yellow atrophy, phosphorus poisoning, and eclampsia; leukemia,

indeed, often spontaneously show this condition when apparently otherwise normal. "Peptone" injections in dogs and guinea-pigs have failed to cause a similar cirrhosis, and hence the value of these and all other rabbit experiments on cirrhosis of the liver is very questionable; however, the possibility of the correctness of the original conclusion still remains open.

¹ Sacconaghi (Zeit. klin. Med., 1903 (51), 187), however, claims to get precipitins by immunizing against either albumose or peptones, which precipitins are not specific for the substance used in immunizing, but are specific for the proteids of the species from which the albumoses and peptones are derived.

² Jour. of Physiol., 1905 (32), 171.

³ Halliburton, *ibid.*, 1905 (32), 174.

⁴ They may be partly hydrolized into smaller complexes, however, primary proteoses being partly changed to deuteroproteoses, and the latter partly to peptones (Chittenden, Mendel, and Henderson, Amer. Jour. Physiol., 1899 (2), 142).

⁵ See Yarrow, Amer. Med., 1903 (5), 452; Ury and Lilienthal, Arch. f. Verdauungskr., 1905 (11), 72; Senator, International Clinics, 1905 (4, series 14), 85.

⁶ Arch. f. exp. Path. u. Pharm., 1905 (54), 88.

especially under x -ray treatment; absorption of simple and inflammatory exudates; and ulcerating pulmonary tuberculosis.¹

It is possible that some of the symptoms of these conditions are due to intoxication with proteoses, for 0.07 to 0.1 gram deutero-albumose will cause a febrile reaction in a healthy man,² but probably their amount is usually too small to cause appreciable effects.³ It is well known, however, that the characteristic rise of temperature following the injection of tuberculin into tuberculous individuals is also produced if minute quantities of proteose solutions are injected in place of tuberculin; therefore, proteoses arising from autolysis in tuberculosis may be of importance in causing fever and other symptoms.⁴

The so-called "Bence-Jones albumose" that appears in the urine of patients with multiple bone-marrow tumors is not a true albumose, but is more closely related to the simple proteids, and is discussed under the head of "Chemistry of Tumors," pp. 427-430.

III. PRODUCTS OF PUTREFACTION AND FERMENTATION⁵

We may perhaps gain some appreciation of the enormous amount of bacterial action that goes on in the normal intestinal digestive processes by considering the fact that as much as one-third of the total weight of the solids of normal feces may consist of bacteria (Strasburger), their proportion being increased in diarrheal disorders and decreased in constipation. They attack all food-stuffs, and among the decomposition-products formed through their activity are undoubtedly many of considerable toxicity. Most of the products of intestinal putrefaction that have as yet been isolated are, however, not extremely poisonous; but many of them are toxic to some degree, and their long-continued absorption may well lead to serious disturbances. Considering them first according to their origin and chemical nature, we take up first the products of:

¹ See Parkinson, *Practitioner*, 1906 (76), 219.

² See Matthes, *Arch. exper. Path. u. Pharm.*, 1895 (36), 437.

³ In a series of unpublished experiments I was unable to cause amyloid degeneration in rabbits by protracted intoxication with proteose solutions.

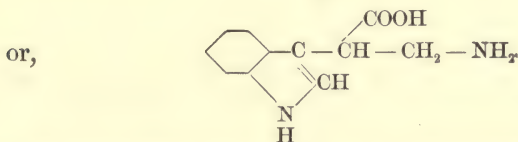
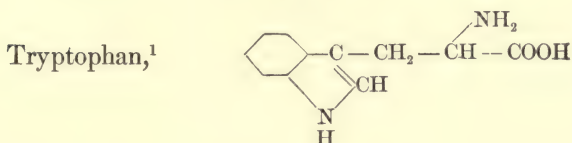
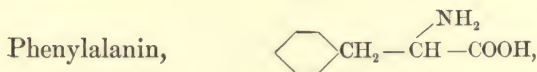
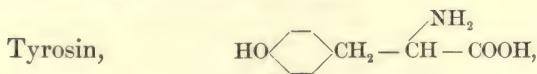
⁴ Simon, *Arch. exp. Med.*, 1903 (49), 449. Concerning relation of tuberculin to proteoses see review by Jolles in Ott's "*Chemische Pathol. der Tuberculose*."

⁵ Complete bibliography given in the résumé on "Intestinal Putrefaction" by Gerhardt, *Ergebnisse der Physiol.*, 1904 (III, Abt. 1), 107.

A. PROTEID PUTREFACTION

(1) SUBSTANCES DERIVED FROM THE AROMATIC RADICALS OF THE PROTEID MOLECULE

In the proteid molecule are contained the following amino-acids with an aromatic nucleus :



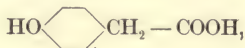
In the intestinal contents have been found a number of substances that are undoubtedly derived from these aromatic radicals. They are (1) *phenol*,



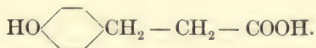
which is formed in small quantities, presumably from tyrosin, as also is the closely related (2) *paracresol*,



and also (3) *para-oxyphenyl acetic acid*,

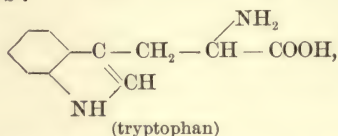


and (4) *para-oxyphenyl-propionic acid*,

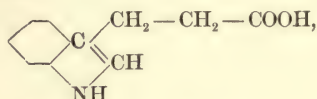


¹The correct structural formula for tryptophan has not yet been finally determined, but the two formulæ given above are as proposed by Ellinger (Ber. deut. Chem. Gesellsch., 1904 (37), 1801). The formula originally proposed by Hopkins and Cole cannot be considered as correct in view of Ellinger's observations. For a full discussion of this subject see Mann's "Chemistry of the Proteids," p. 51.

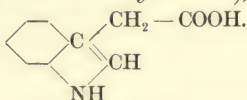
From the tryptophan are formed numerous important substances, as follows :



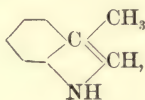
readily yields, through splitting off the NH_2 group and addition of H, *indol propionic acid* (formerly incorrectly called *skatol acetic acid*), which is



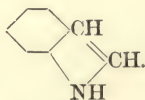
and from which in turn may readily be formed *indol acetic acid* (erroneously called *skatol carboxylic acid*), which is



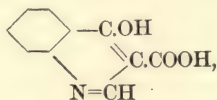
Both of these substances have been found in the intestinal contents. From these substances are formed the better known *skatol*,



and *indol*,



In dogs, but not in man, *kynurenic acid*,

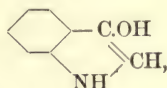


is also formed from tryptophan.¹

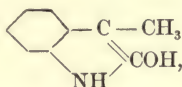
The greatest interest concerning these bodies arises from the fact that after they are absorbed from the intestine they become combined with sulphuric or glycuronic acid, and are excreted in the urine as salts of these acids; consequently the amount of sulphuric acid appearing in the urine in such organic combination ("ethereal sulphuric acid") is considered as an index

¹ See Ellinger, Zeit. physiol. Chem., 1904 (43), 325.

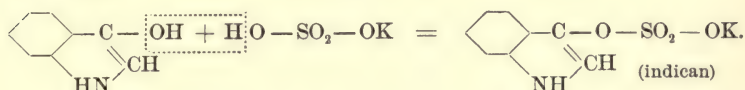
of the amount of intestinal putrefaction. In the case of indol and skatol, which have no hydroxyl group, a preliminary oxidation occurs, whereby indol is converted into *indoxyl*,



and skatol into *skatoxyl*,



and they are then combined with sulphuric or glycuronic acid, as follows :



By far the greater part of these aromatic substances, when excreted in the urine, is combined with sulphuric acid, and but a small part with glycuronic acid; but in case the amount of sulphuric acid available is too small to combine with all the aromatic radicals entering the blood, a large amount of the glycuronic acid compound appears in the urine (*e. g.*, after therapeutic administration of phenol, cresol, thymol, camphor, etc.). Both the preliminary oxidation and the combining with acids seem to occur chiefly in the liver, this process constituting one of the most important of the many protective offices of that organ, since the resulting compounds are much less toxic than are the original substances. Herter and Wakeman¹ have shown that living cells have the power of acting upon indol and phenol (and presumably upon the rest of this group) in such a way that they cannot be recovered by distillation. Most active in this respect is the liver, then in order come kidney, muscle, blood, and brain. The change seems to be a loose chemical combination with the protoplasm of the cells, and the power of the tissues to bring about this combination is not greatly decreased by serious pathological changes in the organs (*e. g.*, ricin poisoning²).

Indol.—This is probably the most important member of this group of substances, the striking color of its derivatives making its detection in the urine easy, so that it is generally used as the

¹ Jour. Exper. Med., 1899 (4), 307.

² For further discussion of this topic, see "Chemical Defences against Poisons of Known Composition," Chapter vii.

most available index of the amount of putrefaction that is occurring in the intestines. The greatest quantities are found when intestinal putrefaction is marked, especially in intestinal obstruction involving the small intestine; obstruction of the large intestine, as Jaffe first demonstrated, does not cause marked indicanuria unless the stagnation involves the ileum, as it may in the latter stages of obstruction. There can be no question that the indican of the urine is derived, at least in part, from the indol formed in the intestine, for administration of indol by mouth to either animals or man causes a considerable increase in the indican present in the urine; however, but 40 to 60 per cent. can be recovered in this way, the rest apparently being oxidized to other compounds, part of which may also appear in the urine.¹ Whether part of the urinary indican is derived from tryptophan liberated during intracellular proteid metabolism, and not from intestinal putrefaction, has long been a disputed point among physiological chemists.² The demonstration by Ellinger and Gentzen³ that tryptophan, when fed or injected subcutaneously causes no increase in urinary indican, whereas its injection into the cecum causes much indicanuria, would indicate that indol is formed from tryptophan only through putrefaction, and not in cellular metabolism. Other experiments support the same view.⁴ However, it is possible that part of the indican present in the urine during conditions associated with gangrene, putrid cancers, putrid placentas, or putrid purulent exudates may be derived from these decomposing materials.

Probably the chief agent in the formation of indol in the intestines and in putrid tissues is the colon bacillus, which, as is well known, produces indol in ordinary culture-media.

Toxicity of Indol.—Although the toxicity of indol seems to be relatively slight, and this toxicity is further reduced by the conversion of indol into indoxyl and indican, yet Herter⁵ found that administration to healthy men of indol in quantities of 0.025 to 2 grams per day caused frontal headache, irritability, insomnia, and confusion; the continued absorption of enough indol to cause a constant strong reaction for indican in the urine is sufficient to cause neurasthenic symptoms. Lee⁶ has also

¹ If gelatin is substituted for proteids in the dietary, indican is not excreted, because gelatin does not contain tryptophan (Underhill, *Amer. Jour. Physiol.*, 1904 (12), 176).

² Literature by Gerhardt, *Ergeb. der Physiol.*, 1904 (III, Abt. 1), 131.

³ Hofmeister's Beitr., 1903 (4), 171.

⁴ See Scholz, *Zeit. physiol. Chem.*, 1903 (38), 513; Underhill, *loc. cit.*

⁵ New York Med. Jour., 1898 (68), 89.

⁶ Jour. Amer. Med. Assoc., 1906 (46), 1499.

demonstrated that indol, skatol, and methyl mercaptan cause muscles to react to stimuli like fatigued muscles. Normal urine contains but about 12 milligrams of indican per day, which amount is so insignificant in proportion to the above-mentioned doses that were found necessary to produce symptoms, that we may well doubt the occurrence of noticeable intoxication from this substance under ordinary conditions. Nesbitt¹ states that twenty times as much indol or skatol as are excreted daily by an adult man may be injected into the jugular vein of a dog of four kilos without causing appreciable effects. Richards and Howland, however, have recently demonstrated the possibility that defective oxidation of substances of this group may permit of intoxication.²

Other Aromatic Compounds.—Skatol seems to accompany indol in small amounts, but apparently in no constant quantitative relation. Although formed in larger quantities in the intestines, it is but slightly absorbed.

Indol-acetic acid appears in the normal urine in extremely minute quantities, and is increased in the same conditions as are indol and skatol.

Phenol appears in the urine normally in very minute quantities—from 0.005 to 0.07 grams per day, according to various observers. Much more is undoubtedly formed in the intestines, for but a small fraction of phenol given by mouth (2 to 3 per cent., according to Munk) appears in the urine as a sulphuric-acid compound; part of the rest is oxidized to hydrochinon and pyrocatechin, $C_6H_4(OH)_2$, and eliminated as ethereal sulphates. The largest quantities are found in the same conditions as indican, except, of course, in "carbolic-acid" poisoning, when the amounts may be so great that practically all the sulphuric acid in the urine is in this organic combination, much of the phenol under these conditions being also combined with glycuronic acid.³

Cresol (chiefly paracresol), *para-oxyphenyl acetic acid*, and *para-oxyphenyl propionic acid* appear under similar conditions, except that the two oxy-acids are possibly also formed within the body through cellular metabolism, as they have been found present in the urine independent of intestinal putrefaction. Probably part of the benzoic acid that appears in the urine combined with glycocoll, as hippuric acid, is derived from intestinal putrefaction.⁴

¹ Jour. Exper. Med., 1899 (4), 5.

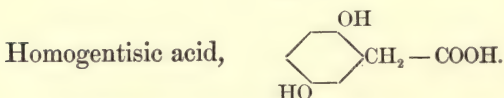
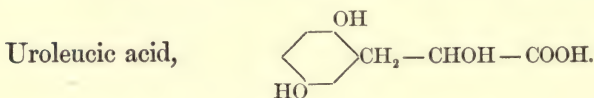
² See note in Science, 1906 (24), 979.

³ See the observations of Wohlgemuth and of Blumenthal (Biochem. Zeitschrift, 1906 (1), 134), on the detoxication of lysol and similar poisons.

⁴ See Prager, Med. News, 1905 (86), 1025; Magnus-Levy, Münch. med. Woch., 1905 (52), 2168.

ALKAPTONURIA¹

Alkaptonuria may be appropriately considered in this connection, since it depends on an abnormal metabolism of the aromatic groups, tyrosin and phenylalanin, which are, partly at least, split out of the proteid molecule in the intestine. This condition is characterized by the tendency of the urine to turn dark on exposure to air, due to the presence in it of two aromatic substances, *homogentisic* and *uroleucic* acids.² It is of rare occurrence, persists throughout life with but little apparent effect upon the health of the individual, and is often hereditary, being grouped by Garrod³ along with cystinuria and albinism as a "chemical malformation" of hereditary origin. The relation of these aromatic bodies to the aromatic constituents of the proteids is best shown by comparing their structural formulæ:⁴



Apparently the condition depends upon an abnormality in the intermediary metabolism, and not upon an abnormal formation of homogentisic acid through intestinal putrefaction, as was at first believed. This abnormality consists not in the excessive formation of homogentisic and uroleucic acids, but in a lack of ability on the part of the alkaptonuric individual to split open

¹ Résumé and literature by Falta, Biochem. Centralblatt, 1904 (3), 174, and Deut. Arch. klin. Med., 1904 (81), 231. Also see Abderhalden, "Lehrbuch der Physiologischen Chemie," Berlin, 1906, pp. 294-298.

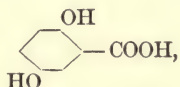
² It should be mentioned that *hydrochinon*, when present in the urine (usually after ingestion of large quantities of phenol), may also turn dark on exposure to air; and *melanin* may be excreted as a chromogen which turns dark on exposure, by patients with melanotic tumors or ochronosis (*q. v.*).

³ Lancet, 1902 (ii), 1616.

⁴ Concerning the formation of homogentisic acid from tyrosin in plant tissues, see Schulze and Castoro, Zeit. physiol. Chem., 1906 (48), 396.

the benzene ring. Tyrosin and phenylalanin seem normally to first suffer a splitting out of the nitrogen radical from the alanin side-chain, and then to be oxidized into uroleucic and homogentisic acids, following which changes comes a disintegration of the benzene ring, with subsequent complete oxidation. The alkaptonuric accomplishes the conversion into the oxy-acid, but the process stops there. Consequently the administration of tyrosin or phenylalanin, or of tyrosin-rich foods—*i. e.*, proteids—causes a marked increase in the amount of homogentisic acid eliminated in the urine (uroleucic acid, which is the precursor of homogentisic acid, has been observed abundantly in but one case); indeed, this increase is almost quantitative. Normal individuals when given these substances, or homogentisic acid itself, destroy them completely, so that the latter does not appear at all in the urine. If alkaptonurics are kept without proteid food for some time, the elimination of alkaptonuric acids goes on, although in diminished amounts, indicating that the aromatic amino-acids formed in tissue katabolism also fail to be destroyed and, therefore, appear in the urine as these derivatives.

As *gentisic acid*,



when given by mouth, is also eliminated unchanged by alkaptonurics, although completely destroyed by normal individuals, it seem evident that the difficulty in metabolism affects the benzene ring itself, and does not depend upon the character of the side-chain. Normal organisms seem to be capable of destroying only such aromatic compounds as pass through a stage of homogentisic acid in being oxidized, which indicates that the benzene ring can be broken up only when oxidized in this particular manner (the 2, 5 position); the alkaptonuric differs in being unable to break up even this form (Falta).

In some cases of alkaptonuria a pigmentation of the cartilages also occurs, *ochronosis*, but the association is not constant; ochronosis may occur without alkaptonuria, and conversely. (See "Ochronosis," page 397.)

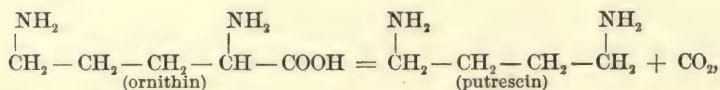
(2) SUBSTANCES ARISING FROM THE FATTY ACID RADICALS (AMINO-ACIDS) OF PROTEIDS

As stated in the introductory chapter, the proteid molecule consists of a combination of a great number of organic acids, of various sorts, all of which have as a common characteristic

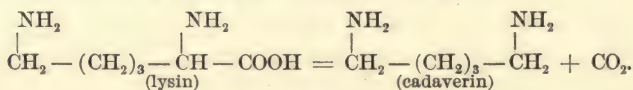
the presence of an NH_2 group attached to the carbon atom nearest the acid radical, the α position; thus, $\text{R}-\text{CHNH}_2-\text{COOH}$. A few of the amino-acids contain an aromatic group, and the relation of these to intestinal decomposition has been considered above. The greater number have a simple fatty acid radical (the simplest amino-acid being glycocoll, $\text{CH}_2\text{NH}_2-\text{COOH}$), and from them are derived by intestinal putrefaction substances that are, for the most part, chemically simple and, as far as known, pathologically unimportant.

Fatty acids may readily be formed from them by splitting out of the NH_2 group; thus acetic acid may be formed from glycocoll, propionic acid from alanin, etc. Apparently butyric and acetic acid are the acids most commonly formed in this way. Gaseous derivatives, such as hydrogen, ammonia, carbon dioxide, and marsh-gas are also produced. *Acetone* is perhaps formed from these fatty acids; it is often present in the intestinal contents, but may come from other sources.

Diamins.—Of more interest are the substances that are formed from the amino-acids by bacterial action, which still retain their nitrogen radicals—the *ptomaines*. Two of these, the diamins *putrescin*, $\text{NH}_2(\text{CH}_2)_4\text{NH}_2$, and *cadaverin*, $\text{NH}_2(\text{CH}_2)_5\text{NH}_2$ are of particular interest¹ because they have been observed in the feces and urine of persons with *cystinuria*. The stools in cholera also seem to contain these ptomaines frequently. Their etiological relation to the cystinuria is no longer accepted, however, and their toxicity is slight. They are probably derived from the diamino-acids of the proteid molecule, putrescin being closely related to *ornithin*,² and is probably formed from it as follows:



while cadaverin is probably formed from *lysin*,



Ethylidendiamin, $\text{CH}_3\text{CH} \begin{array}{l} \text{NH}_2 \\ \text{NH}_2 \end{array}$ which is somewhat toxic,

¹ For discussion of formation and properties of these two ptomaines, see Vaughn and Novy's "Cellular Toxins."

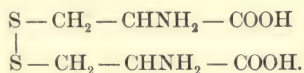
² Ornithin forms part of the arginin molecule, which is the most universally present of all the amino-acids, ornithin being formed when urea is split from arginin.

has also been detected in the contents of the gastro-intestinal tract.

Apparently these substances are absent from normal feces, but this does not exclude the possibility of their normal formation, absorption, and destruction. There is no evidence that they ever cause symptoms or pathological alterations.

(3) SUBSTANCES ARISING FROM THE SULPHUR-CONTAINING RADICAL OF THE PROTEID

Most if not all of the sulphur in the proteid molecule seems to be contained in the amino-acid, *cystin*, which has the following composition :



From this is formed the *hydrogen sulphide* of the intestinal gases, of which about 0.058–0.066 gram is present in each one hundred grams of normal colon contents. Although Senator has described a case in which an intoxication with H_2S of intestinal origin occurred, this gas seems not to be a frequent cause of intoxication, and Senator's case stands almost alone. Under normal conditions H_2S does not appear in the urine, any that may be absorbed probably being oxidized to SO_4 . If enough H_2S should enter the blood so that it was not completely destroyed, it might well cause harm, for it is decidedly toxic, particularly affecting the nervous system; but we have no evidence that this often happens. Van der Bergh¹ has observed cases of intestinal obstruction in which the presence of *sulphemoglobin* in the patient's blood was demonstrated.

Methyl mercaptan, CH_3SH , has also been found in the feces, although it seems not to be abundantly or constantly present, according to Herter,² who found also that mixed bacteria from normal feces rarely produce mercaptan in cultures. However, bacteria from the feces of persons suffering with various diseases often produce mercaptan. *Ethyl mercaptan*, $\text{C}_2\text{H}_5\text{SH}$, and *ethyl sulphide*, $\text{C}_2\text{H}_5 - \text{S} - \text{C}_2\text{H}_5$, have also been described as fecal constituents. It is not known that the mercaptans are a cause of intoxication.

¹ Deut. Arch. klin. Med., 1905 (83), 86.

² Jour. Biol. Chem., 1906 (1), 421.

CYSTIN AND CYSTINURIA¹

The presence of cystin in the urine has been observed in a number of cases, and when present at all it is usually present in considerable quantities. Because of its slight solubility it appears as a deposit of hexagonal crystals, and frequently forms *cystin concretions* (*q. v.*) in the urinary bladder.² Baumann and others observed that in cystinuria the urine often contains, besides the cystin, the diamins *cadaverin* and *putrescin*, which are both formed in the intestines through putrefaction, and they naturally suspected that cystin arose in the same way. Another view was that the diamins interfered with the oxidation of sulphur in the body, so that it was eliminated in the unoxidized form of cystin. But it has been demonstrated that neither of these hypotheses is correct, for (1) cystin could not be found in the feces; (2) if given by mouth, it is completely oxidized, and causes only the appearance of excessive amounts of sulphates in the urine; (3) cystinuria has been observed to occur independent of the presence of the diamins,³ and not to be modified or caused by their administration or pathological formation. The view now prevalent is that the cystin that escapes in the urine in cystinuria is not derived from intestinal putrefaction, but is formed in the tissues from the proteid molecule, and fails to be further decomposed because of some anomaly of metabolism. This view is supported by the fact that cystinuria often appears to be an hereditary disease, occurring in families for several generations; it is independent of the diet, cystin appearing even if proteids are withheld, and also independent of intestinal putrefaction. It having been found that *leucin* and *tyrosin* may also occur in the urine in cystinuria,⁴ it seems probable that this condition depends upon a general abnormality of proteid metabolism.⁵

¹ Literature concerning cystin given by Friedmann, *Ergebnisse der Physiol.*, 1902 (I, Abt. 1), 15; and by Mann, *Chemistry of the Proteids*, pp. 56-64. Cystinuria reviewed by Bödtker, *Zeit. physiol. Chem.*, 1905 (45), 393.

² Abderhalden (*Zeit. physiol. Chem.*, 1903 (38), 557) has described a case in a child in which the organs were infiltrated with masses of the cystin crystals.

³ See Garrod and Hartley, *Jour. of Physiol.*, 1906 (34), 217.

⁴ Abderhalden and Schittenhelm, *Zeit. physiol. Chem.*, 1905 (45), 468.

⁵ The question as to the identity of proteid cystin and "stone cystin," raised by Loewi and Neuberg seems to have been decided affirmatively. As to the condition of general proteid metabolism in cystinuria, the discussion is at the present time too unsettled to permit of consideration. For discussion and literature see Alsberg and Folin, *Amer. Jour. Physiol.*, 1905 (14), 54; also Marriott and Wolf, *Amer. Jour. Med. Sci.*, 1906 (131), 197; Garrod and Hartley, *loc. cit.*

The relation of the diamins to the condition is, however, very uncertain. Cystin does not seem to exert any toxic effect, and patients with cystinuria do not usually appear to suffer greatly from the abnormal metabolism, the chief trouble observed being due to the formation of the concretions in the bladder. Sometimes in children, however, emaciation and early death, without other apparent cause, have been observed, and may be due to the metabolic anomaly.

B. PRODUCTS OF FERMENTATION OF CARBOHYDRATES

These include practically all the members of the fatty acid series, from *formic acid* to *valerianic acid*; and the oxy-acids, *lactic*, *succinic*, and *oxybutyric*; also, *oxalic acid*, *acetone*, *ethyl alcohol*, and the following gases, CO_2 , CH_4 , H_2 . For the most part, the various organic acids are absorbed through the intestinal walls, and are oxidized completely in the tissues without causing any harm whatever. The possibility that acid intoxication may be produced in this way has been suggested, but it is generally believed that this does not occur, except possibly in infants. *Lactic* and *butyric* acids are formed particularly in gastric fermentations in persons with deficient hydrochloric acid, motor insufficiency, or organic obstruction. Most of the disturbances observed in these conditions seem to be due to distention of the stomach with gas, chiefly CO_2 , which is formed during the fermentation. It is possible, however, that the organic acids exercise some irritant effects on the mucous membrane; and they may also cause diarrhea, lactic and acetic acid often being present in diarrheal discharges due to excessive feeding with carbohydrates (Herter).

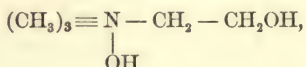
These acids or their salts do not appear in the urine, unless possibly as minute traces, except the *oxalic acid*. Minute quantities (0.02 gm. per day) of this substance are present in normal urine, but larger quantities (*oxaluria*) seem to depend either upon the taking of food containing much oxalic acid (rhubarb, spinach, etc.) or upon excessive gastric fermentation of carbohydrates (Baldwin¹), and perhaps upon excessive destruction of purin bodies, from which oxalic acid may be derived. Probably the small quantities of oxalic acid thus formed do not cause toxic effects, and are important chiefly as causing urinary concretions of calcium oxalate, although there is evidence that long-continued excretion of oxalic acid may cause renal lesions. (See also consideration of oxalate calculi, pages 382, 383.)

¹ Jour. Exp. Med., 1900 (5), 27.

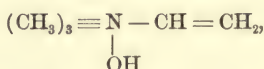
C. PRODUCTS OF THE DECOMPOSITION OF FATS

These differ but little in nature from the products of carbohydrate fermentation, the large fatty acid molecules being broken down to smaller ones. In infants these fatty acids have been believed to be a cause of acid intoxication and acetonuria,¹ but probably they are seldom, if ever, of pathological importance.

It is quite otherwise with the products of decomposition of *lecithin*.² From its molecule is split off the ptomain *cholin*,



which is easily oxidized into a highly poisonous compound, isomeric with *muscarin*, or by losing a molecule of water it forms *neurin*,



which is also very poisonous (discussed under "Ptomaïns," Chap. iv). It has been demonstrated by Nesbitt³ that in the contents of obstructed intestines of dogs that have been fed lecithin-rich food (eggs) both cholin and neurin may be found free, and Kutscher and Lohmann⁴ have found neurin in human urine. It seems possible that some of the toxic effects observed after eating excessively of such foods as calves' brains, or eggs, may depend upon intoxication with the products of lecithin decomposition.

RESULTS OF GASTRO-INTESTINAL INTOXICATION

As we have seen from the above, but few of the known products of gastro-intestinal putrefaction are toxic to any considerable degree, and these are probably produced in too small quantities to cause any appreciable effect, especially in view of the detoxicating and eliminatory powers of the liver, kidney, and other organs. And yet we have abundant clinical evidence that excessive intestinal putrefaction or retention of the intestinal contents cause marked disturbance in health. The slight malaise, headache, and lassitude observed as the result of simple constipation may possibly be adequately accounted for by

¹ Meyer and Langstein, *Jahrb. f. Kinderheilk.*, 1906 (63), 30.

² Literature given by Halliburton, *Ergebnisse der Physiol.*, 1904 (4), 24.

³ *Jour. Exp. Med.*, 1899 (4), 1; see also Hoesslin, *Hofmeister's Beitr.*, 1906 (8), 27.

⁴ *Zeit. physiol. Chem.*, 1906 (48), 1.

intoxication with indol and similar substances, although we have no conclusive proof that such is the case. But the violent effects that follow complete occlusion of the intestine, especially in the upper portion, must be due to some highly toxic substance or substances. The clinical features of obstructive ileus, namely, vomiting, collapse, complete muscular relaxation, and subnormal temperature, are associated with the excretion of large quantities of indican and other substances combined with sulphuric acid, proving that intestinal putrefaction is active. Undoubtedly in ileus we have a profound and rapidly fatal intoxication with substances formed in the obstructed intestines, but as yet we have not isolated any substances from the alimentary canal that possess sufficient toxicity to account for such an intoxication, except possibly the derivatives of cholin, and these are probably formed in too small amounts to account for the conditions observed. Two explanations may be suggested: One is that the intestinal flora becomes altered because of the changed conditions, and bacteria thrive that produce specific soluble toxic substances, analogous to those formed by *B. botulinus*, or similar to the *tyrotoxicon* (Vaughan) that may be formed in milk and milk products. Thus Clairmont and Ranzi¹ found heat-resistant toxic substances in the intestinal contents in ileus (experimental), and similar substances could also be obtained by growing cultures of the intestinal contents on bouillon. Another explanation is that many unidentified poisonous substances are produced in the alimentary canal which ordinarily are destroyed, but under certain conditions may be reabsorbed. That unrecognized toxic substances are formed in the intestines is almost certain, for it has been repeatedly shown that extracts of the contents of the alimentary canal are very poisonous. Although the technic of many of these experiments has been questionable, the results have so often been obtained as to render it probable that the main contention is correct.² Thus Magnus-Alsleben³ has found in the upper part of the small intestine of dogs (except when on milk diet) a very poisonous substance which kills rabbits by respiratory paralysis, but which is inert when injected into the portal vein.

In any case, correctly or incorrectly, a great number of disease conditions have been attributed to poisons of gastro-intestinal origin, including not only such minor conditions as headache,

¹ Arch. klin. Chir., 1904 (73), 696.

² For example, see Roger and Garnier, Compt. Rend. Soc. Biol., 1905 (59), 388 and 674; 1906 (60), 666.

³ Hofmeister's Beitr., 1905 (6), 503.

malaise, lassitude, etc., but also sciatica, tetany, epilepsy, eclampsia, many forms of dermatitis, various forms of nervous diseases, myxedema and cretinism, chlorosis and pernicious anemia, cirrhosis, nephritis, and arteriosclerosis.¹ While in many cases the severity of these various conditions is apparently augmented by intestinal disturbances, the etiologic relation is not so clear. That long-continued intoxication of intestinal origin may cause serious injury to the tissues is, however, extremely probable. There is much reason for believing that many cases of non-alcoholic cirrhosis are due to this cause; not improbably chronic nephritis, myocarditis, and arteriosclerosis may occasionally be the result of long-continued intoxication from the same source.

Tetany associated with gastric dilatation offers perhaps the strongest case, numerous observers having reported finding a marked toxicity of the stomach contents.² Pineles³ considers that all forms of tetany, whether of gastric origin or following thyroidectomy, are due to one and the same "tetany poison."

The relation of intestinal intoxication to the various anemias, particularly chlorosis and pernicious anemia, has been repeatedly indicated and discussed. Clinical evidence strongly indicates that such a relation exists, and there is no doubt that hemolytic substances may be formed in the alimentary tract,⁴ but that chlorosis and pernicious anemia do depend upon intestinal putrefaction or infection is far from established (see "Anemia," Chap. xi).

It seems highly probable that gastro-intestinal "autointoxication" would be a much more serious matter were it not for the mechanisms of defence possessed by the body, especially in the liver.⁵ For example, Richards and Howland have indicated the increased toxicity of indol when the oxidizing power of the liver is reduced, and Herter and Wakeman have shown the power of the liver to combine indol and thus remove it from circulation. This topic has been discussed more fully elsewhere (Chap. vii).

¹ The relation of gastro-intestinal intoxication to these various diseases is reviewed by Weintraud, *Ergeb. allg. Pathol.*, 1897 (4), 17.

² Bibliography by Halliburton and McKendrick, *Brit. Med. Jour.*, 1901 (i), 1607.

³ *Deut. Arch. klin. Med.*, 1906 (85), 491.

⁴ See Külbs, *Arch. exper. Pathol.*, 1906 (55), 73; also Herter, *Jour. Biol. Chem.*, 1906 (2), 1.

⁵ For discussion and literature see Lust, *Hofmeister's Beitr.*, 1905 (6), 132.

CHAPTER XX

CHEMICAL PATHOLOGY OF THE DUCTLESS GLANDS

DISEASES OF THE THYROID¹

As we have much evidence that the thyroid has a marked influence upon metabolism, and also that it may be of importance in preventing autointoxication, the chemistry of diseases of the thyroid may be appropriately considered in connection with the autointoxications.

THE FUNCTIONS OF THE THYROID

Metabolic Function.—That the thyroid has an important relation to metabolism, especially of proteids, is shown by the following facts :

(1) Administration of the gland substance, or active preparations made from it, to healthy men or animals, causes a greatly increased elimination of nitrogen in the form of urea. This nitrogen comes not only from the food, but also from increased tissue-destruction, as is shown by the loss of weight and strength. An increased destruction of the body fat also occurs, so that thyroid therapy has been found efficient in the treatment of obesity, but often dangerous because of the relatively great amount of tissue-destruction.²

(2) Loss of thyroid tissue, either through operation or disease, greatly reduces both nitrogenous metabolism and oxidative processes. Administration of thyroid preparations under these conditions will bring the nitrogen elimination and the gas exchange back to normal.

(3) Deficient thyroid secretion in young animals prevents their developing normally, the amount of deficiency varying from nearly total lack of development in extreme cretinism to slight grades of defective development (infantilism) or delayed

¹ General literature given by Shaw, "Organotherapy," London, 1905; Richardson, "The Thyroid and Parathyroid Glands," Philadelphia, 1905. For earlier literature see Möbius, Nothnagel's System, vol. 22; Wells, Jour. Amer. Med. Assoc., 1897 (29), 897.

² See Rheinboldt, Zeit. klin. Med., 1906 (58), 425.

maturity.¹ In adult animals, besides decreased metabolism there occur also various trophic changes in the skin and its appendages, an increased amount of mucin-like material in the tissues, and greatly decreased nervous and mental activity. All these conditions are relieved to greater or less degree by administration of thyroid tissue or its preparations. Evidently, therefore, the thyroid exerts an influence upon growth and tissue changes; whether this depends upon its influence upon metabolism, or is an independent and specific function, cannot be determined.

How the thyroid or its secretion modifies metabolism is not yet understood. One is reminded of the effects of kinases upon enzymes and their antecedents, and it may be imagined that the thyroid secretion activates both proteolytic and oxidative enzymes within the cells. Shryver,² indeed, did find that administration of thyroid to dogs for some time before killing them causes their liver tissue to undergo autolysis more rapidly than normal, although Wells³ had been unable to observe any increased amount of autolysis when thyroid extracts acted upon liver tissue *in vitro*.

Detoxicatory Function.—The evidence that the thyroid has for its function the *destruction or neutralization of poisonous substances* formed in metabolism or through intestinal putrefaction is as follows:

(1) After total removal of the thyroid from many species of animals acute symptoms develop that suggest strongly an intoxication; often a typical *tetany* develops, resembling the tetany that is associated with gastric dilatation,⁴ and which, as previously mentioned, is believed to be due to toxic products of gastric putrefaction and fermentation.

(2) After removal of the thyroid, marked changes occur in the blood, there being a severe anemia (as low as 2,000,000 red corpuscles), with some leucocytosis, and there occur structural changes in the blood-vessel walls (Kishi⁵). Cytoplasmic degeneration of the liver, kidneys, and myocardium may also result (Bensen⁶). These effects suggest strongly the presence of poisonous substances in the blood of persons or animals lacking sufficient thyroid tissue.

¹ Literature concerning effect of thyroidectomy upon generative functions given by Caro, Berl. klin. Woch., 1905 (42), 310; and Lanz, Beitr. klin. Chir., 1905 (45), 208.

² Jour. of Physiol., 1905 (32), 159.

³ Amer. Jour. Physiol., 1904 (11), 351.

⁴ See Pineles, Deut. Arch. klin. Med., 1906 (85), 491.

⁵ Virchow's Arch., 1904 (176), 260.

⁶ Virchow's Arch., 1902 (170), 229.

(3) All the effects of thyroidectomy are more marked in carnivorous animals than in herbivora; indeed, the latter may be able to live in fair condition for several years without a thyroid.¹ Administration of meat to thyroidectomized herbivora or omnivora causes a great increase in the symptoms, while thyroidectomized carnivora do much better if kept without meat. Thus, Blum² found that thyroidectomized dogs, which were doing well on a milk diet, developed symptoms of athyreosis immediately they were given meat. This fact has been interpreted as indicating that toxic materials are formed from meat in the intestinal tract, which under normal conditions are neutralized by the thyroid. In support of this view is the observation of Watson³ that a pure meat diet causes in fowls a great hypertrophy of both the thyroid and the parathyroid glands, while in rats hyperplastic changes resembling those of exophthalmic goiter are produced by meat diet. On the other hand, one may well imagine that the so-called autointoxication in athyreosis is not from intestinal putrefaction, but is due to the products of incomplete metabolism of proteids within the tissues, which are destroyed when proteid metabolism is normal, but not when the metabolism-favoring influence of the thyroid is wanting. It should also be added that the presence of specific poisonous substances in the blood or urine of thyroidectomized animals has not been conclusively established.⁴

Relation to Generative Functions.—The hypertrophy of the thyroid that occurs at puberty, during menstruation, and especially during lactation, is possibly in response to an auto-intoxication, but far more probably in response to the increased proteid metabolism. In pregnancy and lactation the maternal thyroid functionates for both mother and offspring, the thyroid of the new-born containing either no iodine at all or but the most minute traces. If the greater part of the thyroid is removed from pregnant bitches, the puppies show a great compensatory hypertrophy of the thyroid (Halsted⁵).

¹ Part of these results may be due to the fact that in some herbivora the parathyroids are so far separated from the thyroid that they are not ordinarily removed in thyroidectomy, whereas in many carnivora complete removal of parathyroids with the thyroids is more likely to be accomplished.

² Virchow's Arch., 1900 (162), 375.

³ Lancet, 1905 (i), 347.

⁴ Remedi (Lo Sperimentale, 1902; abst. in Cent. f. Path., 1903 (14), 695) claims that tetanus toxin and other bacterial poisons, when injected into the thyroid gland, are harmless, which he attributes to a neutralization by the colloid.

⁵ Johns Hopkins Hosp. Rep., 1896 (1), 373; also Edmunds, Trans. London Path. Soc., 1900 (51), 221.

CHEMISTRY OF THE THYROID

Whether the function of the thyroid is the neutralization of toxic substances, or a complementary action upon intracellular metabolism, there can be little question that it owes its action to constituents of its specific secretion, the colloid. Furthermore, the chief, if not the sole, active ingredient of the colloid is the iodine-containing substance first discovered by Baumann in 1896, and called by him *thyroidin* (or *iodothyrein*).

The chemical nature of thyroid *colloid* has been studied particularly by Oswald.¹ He found that all the iodine of the thyroid is dissolved out in physiological salt solution, and that none of it is present in an inorganic form. In the salt solution extract are two proteid bodies: one, precipitated by half saturation with ammonium sulphate, contains all the iodine, and seems to be a globulin; it resembles myosin in being precipitated by weak acids, and it contains an easily separated carbohydrate group. The other, precipitated by saturation with ammonium sulphate (exact limits of precipitation are between 6.4 and 8.2 tenths saturation), is a nucleoproteid, containing 0.16 per cent. phosphorus, but no iodine.

The iodine-containing proteid, called *thyreoglobulin*, seems to be the sole active constituent of the colloid; at least, its administration to animals has the same physiological effects as does the entire colloid (great increase in the urea elimination and decrease in blood pressure in animals, curative effect on myxedematous patients), whereas the nucleoproteid is without these effects. Analysis of the thyreoglobulin from various animals has shown it to be of quite constant quantitative composition except for the iodine, which may vary greatly in amount. Normal human thyreoglobulin (from persons living in non-goitrous districts) had the following percentage composition:

C = 51.85, H = 6.88, N = 15.49, I = 0.34, S = 1.86.

Thyreoglobulin from goitrous districts contains much less iodine (0.18–0.19 per cent.), and from calves born with goiters a thyreoglobulin was obtained that agreed in all respects with normal thyreoglobulin, except that it contained no iodine at all. On the other hand, administration of iodides to patients causes the thyreoglobulin to become rich in organically bound iodine.² From

¹ His work is reviewed in his dissertation, "Die chem. Beschaffenheit und die Function der Schilddrüse," Strassburg, 1900; also see Virchow's Arch., 1902 (169), 444.

² Nagel and Roos (Arch. f. Anat. u. Physiol., 1902, p. 267) found that administration of bromides had no effect upon the amount of iodine in the thyroid, and no storage of bromine takes place. Administration of pilocarpine

these facts Oswald believes that the thyreoglobulin, as first secreted by the glandular epithelium, is free from iodine, and that it combines later with iodine from the circulating blood. As yet it has not been ascertained how the iodine is bound to the proteid. It is well known that large amounts of iodine can be introduced into the proteid molecule, apparently through its substitution for hydrogen in the aromatic radicals (tyrosin, phenylalanin, etc.). Thyreoglobulin is not, however, simply an *iodized proteid*, for the iodized proteids that can be artificially prepared do not possess the physiological activity of the thyreoglobulin; furthermore, the saturated iodized proteids contain generally from 5 to 12 per cent. of iodine, as contrasted with the 0.3 to 0.8 per cent. of thyreoglobulin. Oswald has shown that in thyreoglobulin the iodine is not bound to tyrosin, since this can be removed by tryptic digestion without decreasing the amount of iodine in the rest of the molecule; possibly the iodine is bound to phenylalanin.

By decomposing thyreoglobulin by boiling with 10 per cent. sulphuric acid, a body is obtained containing as high as 14.5 per cent. of iodine; this is the *thyroidine* of Baumann, which gives no biuret reaction, yet is physiologically active. The stability of this active constituent of the thyreoglobulin explains the successful administration of thyroid preparations by mouth. It appears to be absorbed unchanged and, unless enormous doses are given, none appears in the urine (Oswald).

The amount of iodine in the thyroid is greatest in middle age, greater in females than in males, and it is decreased in acute infectious diseases and in tuberculosis, alcoholism, and circulatory disturbances (Aeschbacher¹).

THE PARATHYROIDS

Whether the thyroid has any other function than the formation of thyroidine is as yet unknown. Many claim that the thyreoglobulin does not produce the same physiologic and therapeutic effects as does the entire gland substance, but even that is not definitely decided. Furthermore, it is difficult to distinguish between the effects produced by the parathyroid glands and those due to the thyroid itself. The parathyroids were originally considered as but a form of undeveloped accessory

does not increase the amount of iodine in the thyroid. Coronedi and Marchetti (Rivista di Patologia, 1902) consider that administration of fatty combinations of iodine and bromine may partially compensate for loss of the thyroid.

¹ Mitt. a. d. Grenzgeb. med. u. Chir., 1905 (15), 269.

thyroids (a view still held by some¹), but they are now generally believed to be independent organs of fully as great importance as the thyroid. To their removal are ascribed by many investigators the acute manifestations of athyreosis, while the more chronic changes of myxedema are attributed to the loss of the thyroid.²

MacCallum's studies support this view, for he found the results of parathyroidectomy in dogs very different from the results of thyroidectomy. The most prominent symptoms were muscular twitchings, gradually passing into tetanic spasms, and due to nervous impulse rather than to muscular changes, since they did not appear in muscles from which the nerve-supply had been cut off. Trismus, protrusion of the eyes, and rapid respiration without cyanosis (*i. e.*, air hunger) were also observed, and death usually resulted from exhaustion. Apparently these symptoms are due to some toxic substance which accumulates on account of the absence of the parathyroids, for it was found that simply diluting the dog's blood by withdrawing part of it, and injecting a corresponding amount of salt solution, caused a temporary cessation of the tetanic symptoms; and injections of emulsions of parathyroid checked the symptoms for some time, presumably through neutralizing the hypothetical poisons. Degenerative changes that were observed in the cerebral ganglion-cells also favor the view that some unneutralized toxin is responsible for the symptoms following parathyroidectomy. Of particular importance is the demonstration by MacCallum and Slemmons that parathyroidectomy has practically no effect upon proteid metabolism, in marked contrast to the effect of thyroidectomy.

CHEMISTRY OF GOITER

In connection with his earliest studies of thyroiodin, Baumann observed a great difference in the amount of iodine in the thyroid glands of normal individuals living in goitrous districts, as compared with those living in non-goitrous districts. Thus in Freiburg, a goitrous district, the average weight of the dried thyroid was 8.2 grams, each gram containing 0.33 mg. of iodine, a total of 2.5 mg. of iodine to each gland. Glands from Hamburg averaged 4.6 gm. in weight, containing 0.83 mg. of iodine per gram, a total of 3.83 mg. per gland. Berlin glands weighed 7.4 grams, and contained 0.9 mg. of iodine

¹ Kishi, Virchow's Arch., 1904 (176), 260.

² Full discussion by Richardson, "The Thyroid and Parathyroid Glands," Philadelphia, 1905, pp. 29-40; and MacCallum, Med. News, 1903 (83), 820.

per gram, or a total of 6.6 mg. of iodine per gland. Both of the last-named cities are in districts where goiter is not endemic. The thyroids of young children show the same relative paucity of iodine in goitrous districts, as compared with non-goitrous districts. Wells¹ found that the thyroids throughout the United States contain even larger amounts of iodine than the Berlin glands, averaging 10 to 12 mg. per gland, agreeing with the fact that goiter is comparatively rare in this country.² Monery³ has found for France, as Baumann did for Germany, that the amount of iodine contained in the glands of normal individuals is in inverse proportion to the frequency of goiter in districts in which they live. Oswald, and also Aeschbacher,⁴ however, state that normal thyroids in goitrous districts contain more iodine than thyroids from goiter-free districts.

Chemical analyses of goiters have given extremely variable results, and as yet have not led to any satisfactory explanation of the etiology of this condition. Baumann found that in a series of twelve cases of goiter, in which the average dry weight was 32 grams, the amount of iodine in each gram was but 0.09 mg., but the total amount, 2.6 mg., was about the same as in normal glands of the same goitrous district. However, in two goiters large amounts of iodine were found, namely, 17.5 mg. and 31.5 mg. Wells found that the amount of iodine depended upon the structure, for two hyperplastic goiters contained respectively 8.23 and 8.3 mg. of iodine, or about the amount normal for thyroids in this country, whereas two colloid goiters contained 53.16 and 24.59 mg. of iodine. In an adenomatous goiter the new-growth was found to contain 1.98 mg. of iodine per gram, while the rest of the gland contained but 0.8 mg.; the total amount of iodine was 9.26 mg., or the same quantity as found in normal glands. It would seem that in some cases of goiter hyperplastic changes are required to bring the amount of iodine up to normal, perhaps because of a scarcity of iodine in the food or a defective assimilation. In support of this is the fact that Bruns found that hyperplastic goiters are the form most successfully treated by administration of thyroiodin. Colloid goiters possibly depend upon a deficiency in absorption of the colloid from the follicles, or possibly upon a reduced utilization of the thyroid secretion by the body, although we have no evidence for this.

¹ Jour. Amer. Med. Assoc., 1897 (29), 897.

² It is probable, in view of the higher results obtained by Wells and by Oswald, that the results of Baumann and of Monery are somewhat too low.

³ Jour. Pharm. et Chim., 1904 (95), 288.

⁴ Mitt. a. d. Grenzgeb. Med. u. Chir., 1905 (15), 269.

Oswald obtained different results through analyses of the colloid of colloid goiters, finding that colloid goiters contain a thyreoglobulin that is relatively very poor in iodine; in goitrous calves the thyreoglobulin contained no iodine; in human goiters it contained but 0.07 to 0.19 per cent. of iodine, as against a normal proportion of 0.34 per cent. Administration of iodides to a goitrous patient caused a rise in the proportion of iodine in the colloid to 0.51 per cent., showing that in colloid goiters in goitrous districts the thyreoglobulin is probably poor in iodine because of a lack of iodine for it to unite with, and not because it is of an abnormal nature that prevents its chemical combination with iodine.¹ Possibly this explains the greater iodine content observed in colloid goiters in the United States as compared with colloid goiters observed in goitrous districts. In general, Oswald² found the amount of iodine to vary with the amount of colloid in the goiters, although occasionally goiters with exceptionally large amounts of iodine were found, and the proportion of iodine is not usually so great when the amount of colloid is very large. Simple hyperplastic goiters he found poor in iodine, or free from it if they contained no colloid; however, they were found to contain a thyreoglobulin typical in all respects except an absence of iodine. Presumably in such goiters the little thyroiodine present is contained in the parenchymatous cells. The physiological activity of thyreoglobulin obtained from goiters was found to be the same as that from normal glands, except that it was weaker in direct proportion to the amount of iodine it contained, and, therefore, when iodine-free it was without effect. In colloid goiters the greater part of the weight of the gland, three-fourths or more, is made up of this colloid-poor thyreoglobulin. The fluid contents of cystic goiters may be free from iodine, but if they contain much colloid, iodine will be found, and Rositzky³ found 193 mg. of iodine in 20 c.c. of the jelly-like contents of a thyroid cyst.

It has been frequently suggested that the cause of endemic goiter is a deficiency in the iodine in the food, or in the drinking-water, or in the air of the goitrous district. This is supported by the relative infrequency of endemic goiter in districts on the sea-coasts, where the iodine-containing sea-water is sprayed through the air, and where the inhabitants eat largely of sea-foods. However, there are many exceptions, and it cannot be said that this hypothesis of the etiology of goiter rests on

¹ See Kocher, *Mitt. a. d. Grenzgeb. Med. u. Chir.*, 1905, vol. 14.

² *Virchow's Arch.*, 1902 (169), 444.

³ *Wien. klin. Woch.*, 1897 (10), 823.

satisfactory evidence, particularly in view of the abundant iodine content of many goiters. Epidemics of goiter presumably are the results of an infection with some unknown organism, and possibly the endemic form has a similar cause. There is much evidence, in any event, that whatever the cause of goiter may be, it often is related to the drinking-water.¹ Very probably the causes of colloid goiter and parenchymatous goiter will be found to be different from each other and from the causes of cystic and adenomatous goiters.

MYXEDEMA AND CRETINISM

These conditions depend upon a deficiency of thyroid secretion, whether from operative procedure or from pathological alterations in the organ. Consequently we find evidences of a decreased proteid metabolism, the urine containing a diminished quantity of nitrogen, especially in the form of urea, while ammonia and other forms of nitrogen are relatively excessive. The temperature is usually subnormal. Fat and carbohydrate metabolism seem not to be proportionately affected,² and hence the elimination of CO_2 is relatively high as compared to the nitrogen elimination. Gastro-intestinal disturbances are common, with resulting increase in the amount of indican and ethereal sulphates in the urine. Whether from this cause or from deep-seated metabolic anomalies, there is a decided anemia, and the ability of the corpuscles to combine with oxygen seems to be decreased, so that the arterial blood may contain less oxygen than normal venous blood. It is impossible to say whether the failure of growth and development of the young (cretinism), and the mental and physical torpidity of the adult, are due to an autointoxication from products of intermediary metabolism which accumulate because of the failure of the thyroid to furnish the "stimulus" necessary for their complete destruction, or to a lack of some essential action of the thyroid secretion upon the nervous tissues and the growing cells themselves. Administration of thyroid extract to cretinoid children causes retention of nitrogen and phosphorus, but more strikingly of calcium.³

The myxedematous change in the connective tissues is in the nature of a reversion to the fetal type of tissue, and suggests that the thyroid secretion is necessary for proper cell

¹ See de Quervain, *Mitt. a. d. Grenzgeb. Med. u. Chir.*, 1905 (15), 297.

² Rarely myxedema and diabetes have been observed conjointly (see Strasser, *Jour. Amer. Med. Assoc.*, 1906 (44), 765).

³ See Hougardy and Langstein, *Zeit. f. Kinderheilk.*, 1905 (61), 633.

growth. This effect might be either specific, or depend simply on the effect on proteid metabolism. Horsley¹ describes the appearance of the tissues of animals dying after thyroidectomy as follows: The subcutaneous connective tissue is swollen, jelly-like, bright and shining, and excessively sticky. The same thing is observed in the loose tissue of the mediastinum, about the heart, and in the omentum. The submaxillary and parotid glands are greatly enlarged, and have a semi-translucent, swollen appearance; from the cut surface a sticky, glairy fluid exudes. Apparently the parotid becomes transformed into a mucous gland; likewise the mucous membrane of the alimentary tract is swollen and transparent. Fetal tissues contain normally more mucin than those of adults (0.76 per cent. as against 0.37 per cent. in the subcutaneous tissues, according to Halliburton), and in the early stages of the formation of excessive sub-cutaneous tissue, in myxedema such an increase of mucin may be present. But, under ordinary conditions, the term myxedema seems to be entirely a misnomer, for Halliburton's analyses showed that the skin of myxedematous patients contains quite the same amount of mucin as is present in normal skin.² When the condition is of long standing, the amount of mucin may even be much reduced, because of the development of a fibroid character in the connective tissue. However, in monkeys upon which thyroidectomy had been performed, Halliburton³ found a decided increase in the mucin in the tissues throughout the body, especially in the salivary glands, but also in the skin, subcutaneous tissues, and tendons; and mucin was found in the blood, as shown by the following table:

	Skin and subcutaneous tissue.	Tendon.	Muscle.	Parotid.	Submaxillary.	Blood.
Normal monkey . . .	0.89	0.39	0	. . .	. . .	0
" " . . .	0.9	0.5	0	0	0.1	0
After thyroidectomy—						
55 days	3.12	2.55	0	0.72	6.0	0.35
33 days						trace
49 days	2.3	2.4	trace	1.7	3.3	0.8
7 days	0.45	0.904	0	trace	0.16	trace

¹ Brit. Med. Jour., 1885 (i), 211.

² Jour. of Pathol. and Bact., 1893 (1), 90.

³ Quoted by Horsley, *loc. cit.*

It has been suggested that the thyroid produces an enzyme which destroys mucin, but that such is the case has never been demonstrated. Levin¹ states that mucin is toxic for thyroid-ectomized rabbits, but this is not substantiated by Néfédieff.²

That the thyroid is connected with general growth is shown not only by the thyroid abnormalities present in cretinism, but also by the frequent observation of thyroid defects in conditions of delayed growth and development of less extreme degree (*infantilism*), and the favorable effects of thyroid feeding in many such cases. Also in certain types of short-limbed dwarfs (*chondrodystrophia foetalis*) some thyroid anomaly may have an etiologic bearing, for in such a case, in which the thyroid was histologically greatly altered and quite free from colloid, I could find no trace of iodine.³ On the other hand, the thyroid of a giant which I have analyzed contained 62.9 mg. of iodine, or six times the amount present in normal glands.⁴

EXOPHTHALMIC GOITER

It has by no means been conclusively determined that exophthalmic goiter is due to an intoxication with excessive amounts of thyroid secretion, either normal or abnormal, but there is abundant evidence in favor of this view. Most important is the similarity of exophthalmic goiter to the effects of "hyperthyroidism" or "thyroidismus," produced either experimentally or through overuse of thyroid extract for therapeutic purposes. In thyroidismus there are observed a rapid, weak pulse; greatly increased metabolism, especially of proteids;⁵ increased secretion, especially of perspiration; marked nervousness and irritability, often with mental confusion and delusions; gastro-intestinal disturbances, especially diarrhea; sweating, flushing, tremors, palpitation of the heart, loss of weight, and slightly increased temperature are also often observed, and not rarely typical exophthalmos may appear. These manifestations, which are common to both thyroidism and to exophthalmic goiter, are quite the opposite of the characteristic changes of myxedema, with its general lowering of all metabolic and nervous processes. Furthermore, the histological changes

¹ Med. Record, 1900 (57), 184.

² Vrach, 1901 (22), Oct. 27.

³ Reported by Hektoen, Amer. Jour. Med. Sci., 1903 (125), 751.

⁴ Reported by Bassoe, Trans. Chicago Path. Soc., 1903 (5), 231.

⁵ Metabolism in exophthalmic goiter, see: F. Müller, Deut. Arch. klin. Med., 1893 (51), 401; Scholz, Cent. f. inn. Med., 1895 (16), 1041; Magnus-Levy, Berl. klin. Woch., 1895 (32), 650; Schöndorff, Pflüger's Arch., 1897 (67), 395; Voit, Zeit. f. Biol., 1897 (35), 116; Clemens, Zeit. klin. Med., 1906, Bd. 59.

observed in the thyroid are usually quite the same as those of compensatory hypertrophy, suggesting strongly that the goitrous change of this disease is due to a true hypertrophy, with increased production of the specific secretions. Also speaking strongly in favor of the view that exophthalmic goiter is the result of overactivity of the thyroid is the frequent cure of the disease through removal of a large part of the diseased gland.

Corroborative evidence of the hypersecretion idea, and also of the theory that the normal function of the thyroid is the detoxication of metabolic products, seems to have been furnished by the serum treatment advocated first by Ballet and Enriquez, and later by Lanz, and Burghart and Blumenthal.¹ On the principle that after thyroidectomy the blood should contain an accumulation of those substances which the thyroid normally neutralizes, they injected the serum of thyroidectomized goats into patients with exophthalmic goiter, in the hope that these accumulated substances might in turn neutralize any excessive thyroid secretion. Favorable results were obtained, and it was subsequently found that the milk of thyroidectomized goats possesses the same qualities, and may be administered by mouth; this has led to quite extensive clinical use of this method of treatment, which at the time of writing is in the experimental stage.² Of similar significance are the favorable effects obtained by Beebe³ and Rogers⁴ with a serum made by immunization of animals with the nucleoproteids of the thyroid.

Oswald⁵ found that the thyroid in exophthalmic goiter contains generally a smaller proportion of iodine than normal glands, but with the total amount approximately normal. This was also the result of two analyses that I have made. However, the findings are very inconstant, corresponding with the fact that in some cases of exophthalmic goiter the amount of colloid is abundant (in which case the amount of iodine may be large), while usually the amount of colloid is small, and its highly vacuolated condition in hardened sections suggests that it is of unusually fluid consistency. These results, therefore, indicate nothing either for or against the hypothesis that exophthalmic goiter is due to autointoxication with the secretion of the thyroid.

¹ Deut. med. Woch., 1899 (25), 627. Also Möbius, Münch. med. Woch. 1901 (48), 1853; v. Leyden, Med. Klinik, 1904 (1), 1; Eulenburg, Berl. klin. Woch., 1905 (42), 3.

² Negative testimony as to the value of this treatment given by Heinze, Deut. med. Woch., 1906 (32), 755.

³ Jour. Amer. Med. Assoc., 1906 (46), 484; 1906 (47), 655.

⁴ *Ibid.*, 1906 (46), 487; 1906 (47), 661.

⁵ Virchow's Arch., 1902 (169), 475.

There can be no doubt that the thyroid secretion is capable of causing serious intoxication, for patients who have overused thyroid preparations in the treatment of obesity, skin diseases, etc., have often suffered severely from the symptoms mentioned previously, and, in at least one such case, a diagnosis of exophthalmic goiter was made before the cause of the disturbance was detected. Not infrequently evidences of acute intoxication, sometimes resembling tetany, have followed immediately after operations upon the thyroid, and these have been considered as due to intoxication with the large quantities of thyroid secretion that have escaped from the gland during the operative manipulation. The fact that *amblyopia*, resembling that produced by tobacco, etc., may follow overuse of thyroid preparations¹ is also indicative of their toxicity, as also is the *glycosuria* that may result from thyroid administration.

Even if the hypothesis that exophthalmic goiter is due to intoxication with thyroid secretion is correct, we have no satisfactory explanation of the cause of the hyperactivity of the thyroid. In some cases degenerative changes have been observed in the superior cervical sympathetic ganglia, and cure or improvement of exophthalmic goiter is said to have followed resection of these ganglia; however, this relation has not been observed at all constantly. In other cases there has been evidence that suggested a primary intoxication with the products of intestinal putrefaction, leading to a secondary hyperplasia of the thyroid, but this also seems to be an exceptional observation. All things considered, it seems most probable that the hyperactivity of the thyroid is due to some exciting condition, and is not of itself primary, although the resulting hypersecretion of the thyroid may cause the dominant features of the disease. The frequent association of exophthalmic goiter with puberty and pregnancy suggests that some abnormality in the function of the generative organs may be a frequent starting-point of the thyroid derangement.

The Relation of the Parathyroids to Exophthalmic Goiter.—This has not yet been definitely established. As nervous manifestations are very prominent after parathyroidectomy, so that many experimenters attribute all the acute nervous and muscular symptoms of total thyroidectomy to simultaneous removal of the parathyroids, it has seemed very probable that these organs may be more closely associated with exophthalmic goiter than is the thyroid itself.² Against the

¹ Birch-Hirschfeld and Inouye, Graefe's Arch., 1905 (61), 499.

² This subject is thoroughly reviewed by MacCallum, Med. News, 1903 (83), 820.

hypothesis that exophthalmic goiter is due to parathyroid insufficiency, however, stand the following facts :

(1) Removal of one lobe of the thyroid often causes improvement or recovery in this disease, yet with the lobe of the thyroid is generally removed the adjacent parathyroid, which would decrease the amount of parathyroid tissue, and make worse any existing parathyroid insufficiency. (2) Therapeutic administration of parathyroid tissue or extract has had no significant effect on the disease. (3) No considerable or characteristic anatomical changes occur in the parathyroids in exophthalmic goiter,¹ while the great majority of all cases show hypertrophic changes in the thyroid. (4) The parathyroids seem to have no particular influence on metabolism (MacCallum), while metabolic abnormalities are very marked in exophthalmic goiter.

ACROMEGALY AND THE HYPOPHYSIS

Although in nearly all cases of acromegaly alterations are observed in the hypophysis, yet it has not been conclusively established that the peculiar overgrowth characteristic of this disease, and of giantism, is dependent upon this organ.² A great variety of lesions has been described in the hypophysis of acromegals, adenomatous and sarcoma-like changes having been most frequently observed ; but similar and equally diverse lesions have been observed without acromegaly.³ All the facts taken together, however, point to hyperactivity of the hypophysis as the cause of acromegaly ; in some cases this hyperactivity is associated with gross enlargement, but often the gland shows only histological changes, which consist chiefly of hyperplasia of the chromophile cells of the anterior lobe (Lewis).

Equally contradictory and inconclusive are the results of experimental studies of the normal function of the hypophysis, for, while some observers have described muscular tremors and spasms, emaciation, and many other symptoms after extirpation of the hypophysis, other investigators have observed no effects at all.⁴ Administration of hypophyseal tissue seems to have no characteristic effects upon either metabolism or the nervous system. Thompson and Johnson⁵ found that hypophysis feeding causes loss of weight in dogs and increased elimination of nitrogen and phosphoric acid. Extracts of the anterior lobe

¹ MacCallum, Johns Hopkins Hosp. Bull., 1905 (16), 287.

² See Mitchell and LeCount, New York Med. Jour., 1899 (69), 517.

³ Full literature given by Lewis, Johns Hopkins Hosp. Bull., 1905 (16), 157.

⁴ Friedmann, Berl. klin. Woch., 1902 (39), 436.

⁵ Jour. of Physiol., 1905 (33), 189.

cause a slight fall in blood pressure (Hamburger¹), while the infundibular lobe causes some rise in pressure and slowing of the heart (Howell²).

That the hypophysis is related to the thyroid there can be no question, for changes in one organ are very frequently associated with changes in the other. Thus Pel,³ Pope,⁴ and others have observed the association of myxedema and acromegaly; thyroid enlargement is almost constantly found in acromegaly and giantism; in exophthalmic goiter the hypophysis is often histologically changed (Benda⁵). In some cases of atrophy of the thyroid an increase in the size of the hypophysis is observed, which resembles a compensatory hypertrophy in that a considerable quantity of colloid-like material appears in the gland; this has been described in myxedema by Ponfick,⁶ and in *scleroderma* by Hektoen.⁷ Many observers state that after thyroidectomy a similar compensatory hypertrophy of the hypophysis occurs. Furthermore, the normal hypophysis contains iodine; in fourteen glands that I collected and analyzed the total amount of iodine was 0.05 mg., or an average of 0.0036 mg. for each gland.⁸ The proportion of iodine is about one-fiftieth as much as in the thyroid. It is not known whether the iodine is contained in the form of thyreoglobulin or not, but the fact that the hypophysis may contain colloid, and that it is embryologically of similar derivation to the thyroid, suggests an affirmative answer.

Metabolism in Acromegaly.—Metabolism has been studied in a few cases of acromegaly,⁹ and all investigators have observed a decided retention of nitrogen and phosphorus, corresponding to the growth of the soft tissue; and a less marked retention of calcium, because of overgrowth of bone; an unusually large proportion of the calcium seems to be excreted by the kidneys as compared to the bowels. Excessive excretion of fatty acids without acetone was observed by Edsall and

¹ Amer. Jour. Physiol., 1904 (11), 282.

² Jour. Exp. Med., 1898 (3), 245.

³ Berl. klin. Woch., 1905 (42), No. 44a, p. 25.

⁴ Brit. Med. Jour., 1905 (ii), 1520.

⁵ Arch. Anat. u. Physiol., 1900 (Physiol. Abt.), 373.

⁶ Zeit. klin. Med., 1899 (38), 1.

⁷ Cent. f. Path., 1897 (8), 673. According to the studies of metabolism in *scleroderma* by Bloch and Reitmann (Wein. klin. Woch., 1906 (19), 630) this disease bears a resemblance to thyroid diseases, rather than to gastrointestinal putrefaction, as has been suggested frequently.

⁸ Jour. Amer. Med. Assoc., 1897 (29), 1011.

⁹ Schiff, Wien. klin. Woch., 1897 (10), 277; Moraczewski, Zeit. klin. Med., 1901 (43), 336; Edsall and Miller, Univ. of Penn. Med. Bull., 1903 (16), 143.

Miller. Franchini¹ found that administration of hypophysis tablets had no effect upon the metabolism of an acromegalic. Glycosuria is frequently present in acromegaly, and is also often present in hypophyseal tumors without acromegaly.²

THE ADRENALS AND ADDISON'S DISEASE³

In common with the other ductless glands, the adrenals have been considered by many as having for their chief function the neutralization of poisons of metabolic or gastro-intestinal origin. The evidence in support of this view is, however, by no means conclusive. When the function of the adrenals is reduced through pathological alterations (Addison's disease), or abolished by experimental extirpation, a number of characteristic constitutional changes follow. Most prominent is the profound muscular weakness, which is more marked by early fatigue than by weakness during a single effort. The decreased cardiovascular tone is also striking, and a severe anemia is usually present. Gastro-intestinal disturbance is marked, anorexia, nausea, vomiting, and diarrhea usually occurring. In man, pigmentation of the skin and mucous membranes with a pigment resembling melanin in appearance, is one of the most striking features, and some evidences of pigmentation are occasionally observed after experimental adrenalectomy. The exact nature of this pigment, and the reasons for its accumulation in Addison's disease, are both unknown. (Discussed under "Pigmentation," page 397.) In experimental animals it has been found that the blood is toxic for other animals, which is usually interpreted as meaning that toxic products accumulate, which the adrenals normally neutralize or destroy; but it is equally possible that these toxic substances are produced only after removal of the adrenals, and not in normal metabolism.⁴ The metabolism is decreased, but no characteristic changes are observed (Neusser⁵).

Adrenalin.—Administration of adrenal tissue to either man or animals, while unable to compensate for loss of the glands, has very profound effects. These are due chiefly, if not

¹ Bull. sci. med. Bologna, 1905 (75), 8.

² Launois and Roy, Arch. Gén. de Méd., 1903 (191), 1102.

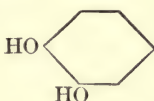
³ General review and literature given by Shaw, "Organotherapy," London, 1905.

⁴ Sergeant (Presse Méd., 1903 (11), 813) describes as characteristic of "acute insufficiency of the adrenals" a certain symptom-complex that simulates a severe intoxication. Hemorrhage into the adrenals often causes acute symptoms resembling a profound intoxication, especially like peritonitis (see Simmonds, Virchow's Arch., 1902 (170), 242; Dudgeon, Amer. Jour. Med. Sci., 1904 (127), 134).

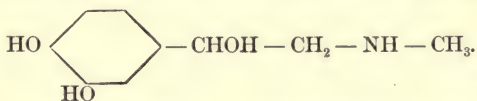
⁵ Nothnagel's System, Bd. xviii, 3 Teil.

entirely, to the presence in the gland of a specific substance with remarkably great power of raising blood pressure by causing general arterial contraction, at the same time causing contraction of all other voluntary muscles that are under control of the sympathetic nervous system.¹ According to Langley² and to Elliot,³ adrenal extract acts upon some receptive substance present in the muscle, which is independent of the nervous system, since the muscles react to adrenalin after the nerves have been sectioned and even after their fibrils and endings have degenerated. Adrenal administration seems to have no marked or constant effect upon metabolism, for most of the results reported in the literature are very contradictory, some observing nitrogen loss and some nitrogen retention.⁴

The active substance has been isolated in pure crystalline form, and although various names have been given to it, *adrenalin* is the one in most general use. As yet unanimity has not been reached concerning the structural composition of adrenalin,⁵ but it is unquestionably related to *pyrocatechin*,



and the formula accepted by the majority of chemists⁶ is



This view of its structure suggests that it is derived from the aromatic groups of the proteid molecule.⁷ Dakin,⁸ starting with pyrocatechin, has synthesized a substance with the same formula as that given, which has physiological effects similar to those of the natural adrenalin, although the synthetic substance differs from the natural in being optically inactive.

Important as this substance is, its production is probably not the sole function of the gland, for the administration of adren-

¹ Moore and Purinton (Amer. Jour. Physiol., 1900 (4), 51) state that the embryo human adrenal has no effect on blood pressure, and does not give the characteristic "chromogen" reaction with ferric chloride.

² Jour. of Physiol., 1905 (33), 374.

³ *Ibid.*, 1905 (32), 401.

⁴ Vollbracht, Wien. klin. Woch., 1899 (12), 737; Pickardt, Berl. klin. Woch., 1898 (35), 727; Kaufmann, Cent. f. Stoffwechsel, 1901 (2), 173.

⁵ See Abel and Taveau, Jour. Biol. Chem., 1905 (1), 1.

⁶ See Friedmann, Hofmeister's Beitr., 1906 (8), 95.

⁷ See Halle, Hofmeister's Beitr., 1906 (8), 276; also Friedmann, *loc. cit.*

⁸ Jour. of Physiol., 1905 (32), p. xxxiv; Proc. Royal Soc., 1905 (76), 491.

alin or adrenal extracts, as before mentioned, will not counteract the loss of the adrenals. Thus, in 97 cases of Addison's disease collected by Adams,¹ treatment with adrenal extract caused some improvement in 31, 43 were not benefited, 7 were made worse, while but 16 were permanently improved. In three cases in which grafting of adrenal tissue was performed, the patients seemed to have been made worse. It is possible that the cortical and medullary portions have different functions, since the latter, which contains most of the adrenalin,² originates in the sympathetic nervous system, while the cortex is formed from the same embryonal tissue elements as the kidneys and the generative glands. In rabbits the cortex hypertrophies during pregnancy; in frogs seasonal variations in structure occur, corresponding to the period of mating; and cases of sexual precocity have been found associated with adrenal hypertrophy, while cases of defective sexual development have been found associated with adrenal atrophy. Therefore, it seems probable that the cortical portion has to do with the generative organs. Karakascheff³ believes, however, that Addison's disease depends upon lesions of the adrenal cortex, since the medullary part may be entirely destroyed without the appearance of the disease. The view that a diseased condition of the semilunar ganglion, or of the entire sympathetic nervous system, is the cause of Addison's disease has been long held by many, and undoubtedly bears some relation to the observation of Langley,⁴ that the effects produced by adrenalin upon any tissue are such as follow excitation of the sympathetic nerve which supplies the same tissue.

The amount of adrenalin secretion seems to be little modified by disease or drugs (atropin), according to Ehrmann,⁵ although in acute experimental infections in animals the amount present in the gland seems to be decreased. Adrenalin is not readily destroyed by postmortem autolysis of the glands.⁶ In chronic insanity Mott and Halliburton⁷ found the adrenals atrophied and deficient in adrenalin; this condition seems to be due to the chronic disease and not specifically related to the insanity, and the authors suggest that defective vascular tone in chronic diseases may be partly dependent upon adrenal atrophy.

¹ Practitioner, 1903 (71), 472.

² See Abelous, *Compt. Rend. Soc. Biol.*, 1905 (59), 520.

³ Ziegler's Beitr., 1904 (36), 401.

⁴ *Jour. of Physiol.*, 1901 (27), 237.

⁵ *Arch. exp. Path.*, 1906 (55), 39.

⁶ See *Gazette degli Osped.*, 1896, No. 12.

⁷ *Jour. of Physiol.*, 1906 (34), p. iii.

Arterial Degeneration from Adrenalin.—An interesting result of repeated injections of adrenalin into animals is the appearance of a marked atheromatous degeneration of the aorta, with calcification. This was first observed by Josué, and since confirmed by Erb, Fischer, Gouget, Loeb and Githens, and many others.¹ These lesions are quite different from those of human arteriosclerosis, the chief change being degeneration of the muscle-cells of the media, without any considerable inflammatory reaction.² They do not seem to be due to the heightened blood pressure, since simultaneous administration of substances that keep the blood pressure down does not prevent the atheroma from developing (Braun), while other substances that raise blood pressure, such as nicotine (Josué) or pyrocatechin (Loeb and Githens), do not cause atheroma. Presumably, therefore, adrenalin causes the arterial changes by a direct toxic action.³ Myocardial degeneration is also observed in experimental animals, and later may lead to an interstitial myocarditis (Pearce⁴). These experiments suggest the possibility that oversecretion of adrenalin may be a cause of arteriosclerosis, but there is no evidence that this actually occurs in man.

Adrenalin Glycosuria.—Another interesting effect of injection of suprarenal extracts or of pure adrenalin is the marked glycosuria that follows. This property, first described by Blum and directly after by Croftan, has been particularly studied in Herter's laboratory, where a number of interesting facts have been developed.⁵ Subcutaneous injections cause less glycosuria than intravenous or intraperitoneal injections, while most minute quantities of adrenalin cause glycosuria if applied directly to the surface of the pancreas.⁶ The glycosuria seems to depend upon an increased conversion of glycogen into sugar in the

¹ Literature given by Loeb and Githens, *Amer. Jour. Med. Sci.*, 1905 (130), 658; by Ellis, *Amer. Med.*, 1906 (11), 292; and by Pearce and Stanton, *Jour. Exp. Med.*, 1906 (8), 74.

² See Klotz, *Jour. Exper. Med.*, Aug., 1906.

³ According to v. Koranyi the production of sclerosis may be prevented by iodine administration (*Deut. med. Woch.*, 1906 (32), 679); see also Cummins and Stout, *Univ. of Penn. Med. Bull.*, July, 1906. Pearce and Baldauf (*Amer. Jour. Med. Sci.*, 1906 (132), 737) suggest that local anemia due to constriction of the vasa vasorum is the cause of the arterial degeneration.

⁴ *Jour. Exp. Med.*, 1906 (8), 400.

⁵ Literature by Herter and Richards, *Med. News*, 1902 (80), 201; Herter, *Amer. Med.*, 1902 (3), 771; Herter and Wakeman, *Virchow's Arch.*, 1902 (169), 479; *ibid.*, *Amer. Jour. Med. Sci.*, 1903 (125), 46; Underhill and Clossen, *Amer. Jour. of Physiol.*, 1906 (17), 42.

⁶ The relation of the pancreas to adrenalin glycosuria is brought into question by the results obtained by Underhill (*Jour. of Biol.*, 1906 (1), 113) and Velich, *Virchow's Arch.*, 1906 (184), 345.

liver,¹ perhaps indirectly through some alteration in the action of the pancreas. Experimental manipulation of the adrenals causes glycosuria, while their removal is followed by a decrease in the amount of sugar; hence it is possible that both in health and in disease the adrenals may have an important influence on carbohydrate metabolism. Iwanoff² has found that adrenalin increases the rate of discharge of sugar from the glycogen-rich liver through which salt solution is being transfused, which observation suggests that possibly adrenalin acts directly upon the glycogen-splitting enzyme of the liver-cells. Underhill and Clossen³ suggest that adrenalin acts upon the liver through the sympathetic nervous system; they believe that adrenalin glycosuria bears no relation to human diabetes.

¹ See Wolownik-Charkow, *Virchow's Arch.*, 1905 (180), 225.

² *Cent. f. Physiol.*, 1905 (19), 891.

³ *Loc. cit.*

CHAPTER XXI

URIC-ACID METABOLISM AND GOUT

THESE subjects have been the object of such a prodigious amount of research that it is far beyond the scope of this work to review the history and the details of the investigations. Such a review is also particularly unnecessary, since it can be found in the works on physiological chemistry and various treatises on metabolism, and also since it has been recently thoroughly covered in English by Barker and by McCrudden.¹ The more recent advances have also been discussed by Chittenden in his Shattuck Lecture,² and by Mendel in his Harvey Lecture.³

Consequently the attempt will be made in this chapter merely to give, as briefly as possible, the views now most generally accepted concerning the nature and metabolism of uric acid, and its relation to pathological processes. For the historical discussion, indicating by what devious steps we have reached our present understanding concerning this long-disputed subject, the reader is referred to the articles mentioned, upon which I have freely drawn. In these articles will be found a complete bibliography to the beginning of 1906.

THE CHEMISTRY OF URIC ACID

It is the very great service of Emil Fischer to have shown us the structure of the uric-acid molecule, the empirical formula of which, $C_5H_4N_4O_3$, had long been known. He demonstrated that it is a member of a group of substances, which are all characterized by being built up about a certain nucleus, C_5N_4 . As the simplest member of the group is a synthetically formed

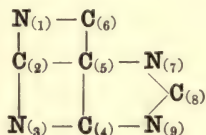
¹ L. F. Barker, "Truth and Poetry Concerning Uric Acid," Chicago, 1905; this will also be found as a series of editorials under the same title in the *Journal of the Amer. Med. Assoc.*, 1905 (44), from Jan. 14 to May 13. F. H. McCrudden, "Uric Acid," New York, 1906. Other complete reviews are given by Wiener, *Ergebnisse der Physiol.*, 1902 (1), 555; *ibid.*, 1903 (2), 377; Burian and Schur, *Pflüger's Arch.*, 1900 (80), 241; 1901 (87), 239; Walker Hall, "The Purin Bodies of Food-stuffs," 1903.

² Boston Med. and Surg. Jour., 1905 (153), 179.

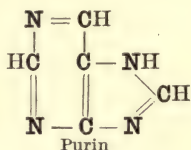
³ *Journal Amer. Med. Assoc.*, 1906 (46), 843.

body, *purin*, the nucleus is called the "*purin nucleus*." The structural relations of the better-known "*purin bodies*" to this purin nucleus and to each other is clearly shown by their structural formulæ, as given below :

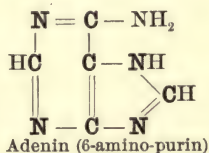
The atoms in the "*purin nucleus*" are arranged as follows :



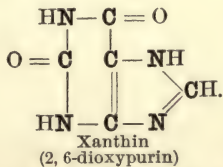
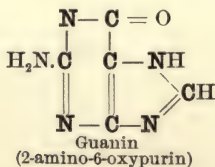
To each atom has been given a number, as shown, for the purpose of facilitating reference to the location of various atoms and groups that are attached to this nucleus. The structure of purin itself is as shown below :¹



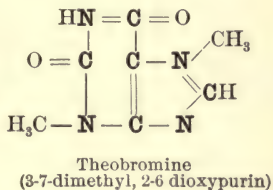
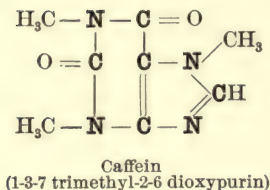
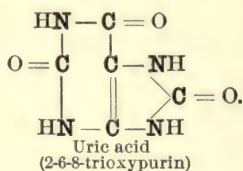
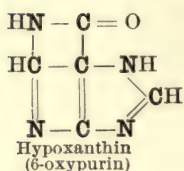
The derivatives of purin are described by stating to which atom of the purin nucleus the combining groups are attached. Thus, adenin is referred to as 6-amino-purin, and therefore has the following formula :



Other important members of this group of "*purin bodies*," (also called *xanthin* bodies, *alloxuric* bodies, and *nuclein* bodies) are built up about the purin nucleus as shown below :



¹ In these formulæ the symbols of the atoms forming the purin nucleus are in heavy type.



Properties of Uric Acid.—Uric acid, when pure, is white, and crystallizes in rhombic tablets. Its solubility is very slight; at room temperature (18°) it dissolves but about one part to 40,000 of water, so that a saturated solution contains but 0.0253 gram to the liter. It is much more soluble in blood-serum, dissolving in 1000 parts,¹ probably held in some complex combination. His and Paul have shown that in a saturated solution only 9.5 per cent. of the molecules are dissociated, the dissociation occurring in two steps; the first and chief dissociation is into H and $\text{C}_5\text{H}_3\text{N}_4\text{O}_3$, which then undergoes further dissociation into H and $\text{C}_5\text{H}_2\text{N}_4\text{O}_3$, the latter dissociation being very slight. If any other acid is present in the solution, its dissociation and liberation of free hydrogen ions interferes with the dissociation of the uric acid, and as the undissociated uric acid is extremely insoluble, the amount dissolved in an acid solution is much less than in a neutral solution.

With alkalis uric acid yields two series of salts, corresponding to these two steps in dissociation: one, in which one atom of the base enters, is called the *biurate* or *monobasic urate*; the other is the so-called “neutral” or *bibasic urate*.² Of the two, the latter is much the more soluble. The monosodium urate forms colloidal solutions in water, from which the crystalline salt gradually falls out.

In the urine the uric acid and the urates are kept in solution by the phosphates, the disodium phosphate preventing the decomposition of the urates into uric acid by the acid salts of the urine. Possibly other constituents of the urine, especially

¹ Taylor, Jour. Biol. Chem., 1906 (1), 177.

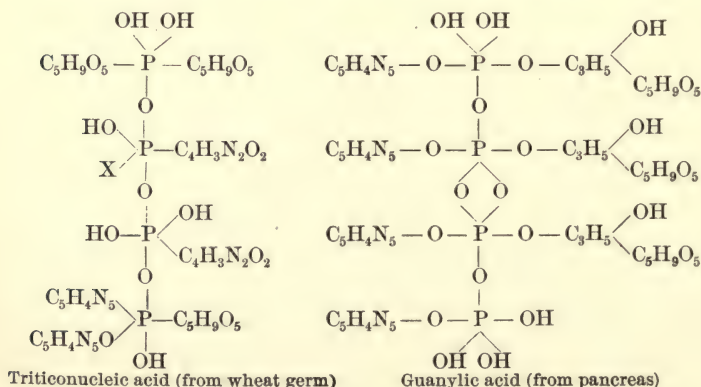
² As a matter of fact, both salts give a slightly alkaline reaction when dissolved in water (Taylor).

the pigments, also aid in its solution. How the uric acid is kept in solution in the blood is not exactly understood, but it seems probable that it is in combination with some organic substance, possibly with some derivative of nucleic acid.

FORMATION OF URIC ACID

The origin of uric acid is chiefly, although not exclusively, from the nucleoproteids, and it is customary to refer to uric acid formed from the nucleoproteids of the foods as "*exogenous*" uric acid, in contrast to the "*endogenous*" uric acid that is formed from the nucleoproteids of the body cells during their catabolism.

This may be readily explained by a brief consideration of the composition of the nucleoproteids. The nucleoproteids may be looked upon as salts formed through combination of proteids with nucleic acid. Nucleic acid in turn is a compound of phosphoric acid with purin bases, pyrimidin bases, and usually also with carbohydrate radicals. For example, the following structural formulæ have been proposed as indicating the composition of certain nucleic acids, showing (provisionally) how pentose radicals ($C_5H_9O_5$) purin radicals ($C_5H_4N_5$ and $C_5H_4N_5O$) and pyrimidin radicals ($C_4H_3N_2O_2$) may be grouped about phosphoric-acid radicals to form various nucleic acids:

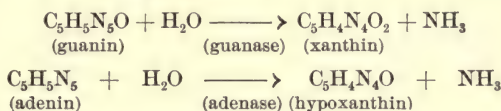


Nucleic acids of different origins differ from one another in the number and variety of purin bases they contain, and also in their carbohydrate radicals, hence an almost infinite variety of nucleic acids and nucleoproteids may exist.

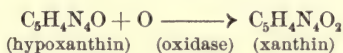
Uric acid itself does not exist in the nucleoproteid molecule,

but it is readily formed from any of the purin bases, and the steps by which it is formed are believed to be as follows :

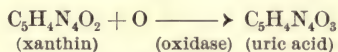
Nucleoproteids when acted upon by trypsin have the proteid group digested away, leaving the nucleic-acid radical unaffected. Probably in intracellular metabolism the proteolytic enzymes of the cell have a similar action upon the nucleoproteids, setting free the nucleic acids, which are then attacked by a specific enzyme (or enzymes) called by Iwanoff "*nuclease*." This enzyme liberates from the nucleic acid the purin bases, of which adenin and guanin are the most abundant.¹ These two substances are in turn acted upon by other specific intracellular enzymes, which, through hydrolysis and liberation of ammonia (*deamidization*), convert them into xanthin and hypoxanthin, as shown by the following equation :



The final step, the conversion of the xanthin and hypoxanthin into uric acid, is accomplished through oxidation by the action of an oxidizing enzyme. First, the hypoxanthin is converted into xanthin :



and the xanthin is then oxidized into uric acid, thus :



Not only are these reactions accomplished in the body during metabolism, but it has been found possible to obtain enzyme-containing extracts from the tissues, which will bring about these various reactions when allowed to act upon pure adenin, guanin, etc., outside the body. Each reaction seems to depend upon a specific enzyme.

Another possible source of uric acid is through synthesis. In birds, which eliminate most of their nitrogen in the form of uric acid, synthesis of uric acid undoubtedly occurs. It would seem possible, therefore, for synthesis of uric acid to occur in mammals, but as yet satisfactory experimental evidence is lacking that such synthesis does occur. The greater part, and perhaps all, of the uric acid is formed in mammals through oxidation of performed purin groups.

¹ See review by Jones and Austrian, *Zeit. physiol. Chem.*, 1906 (48), 110; also full summary by Bloch, *Biochemisches Centralblatt*, 1906 (5), 521.

It should also be mentioned that not all of the purin bases of the body is bound in the form of nucleic acid. A considerable amount is present in a free condition, or at least not bound in nucleic acid, especially in muscle tissue, where much more of the purin bases is free than combined. Uric acid can be formed equally as well from the free purin bases as from purin bases liberated from nucleic acid—indeed, evidence has recently been brought forward indicating that a large proportion of the uric acid arising during metabolism (endogenous) comes from the free hypoxanthin of the muscles.

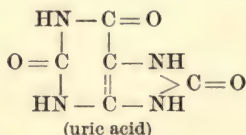
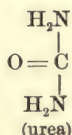
As to the place where uric acid is formed, it seems probable that the purin bases and the necessary oxidative enzymes are present in many if not in all varieties of cells; certainly all the chief visceral tissues and the muscles are capable of forming uric acid.

DESTRUCTION OF URIC ACID

By no means all of the purin bases that is formed in the tissues, or that is taken into the alimentary canal in the food, appears in the urine as uric acid; by far the greater part is destroyed through oxidation, forming urea from the nitrogen-con-

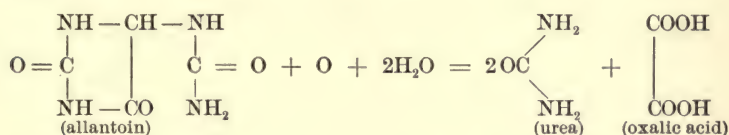
taining groups, and oxalic acid, from the remaining $\begin{array}{c} \text{C}=\text{O} \\ | \\ \text{C} \\ || \\ \text{C} \end{array}$ group,

in case oxidation is not complete. The relation of uric acid to urea can readily be seen by comparing their structural formulæ:



Of the purin bases taken in the food, it is estimated that in carnivora, such as dogs, but one-twentieth to one-thirtieth appears in the urine as uric acid; in herbivora (rabbits), one-sixth; and in omnivora (man), one-half. Apparently the purin bases taken in the food are first converted into uric acid before being destroyed, for it has been found that if uric acid is injected into an animal, the same amount appears in the urine as when a corresponding quantity of purin bases is given to the same animal by mouth or subcutaneously.

The steps by which uric acid is destroyed are not known, except that in any case the nitrogen is eliminated as urea. Experimental decomposition of uric acid in the laboratory shows that it can be split up in at least three different ways. By certain methods it yields glycocoll, ammonia, and carbon dioxide; by another method the products are first, *alloxan* ($C_4H_2N_2O_4$), which later yields *parabanic acid* ($C_3H_2N_2O_3$), and this in turn yields oxalic acid and urea. By still a third method uric acid is decomposed into *allantoin* ($C_4H_6N_4O_3$) and carbon dioxide, and the allantoin yields, on further oxidation, urea and oxalic acid, thus:



It seems quite probable that allantoin is one of the first steps in the metabolic oxidation of uric acid in the body, for if excessive quantities of uric acid or of purin-rich foods are fed to dogs, a large amount of allantoin appears in the urine. As smaller quantities of purins are completely oxidized, it seems probable that the excessive amounts cannot be completely oxidized and are eliminated while in the allantoin stage. Allantoin seldom appears in human urine, except in young infants and pregnant women, in both of which cases the cause may be either deficient oxidation or excessive destruction of tissue nucleins. However, there is also evidence that glycocoll is formed in the tissues from uric acid, and hence it is possible that uric acid may be broken down along more than one of the lines of decomposition indicated above. In any case, however, the destruction of uric acid depends upon oxidation, and is, therefore, but a continuation of the process by which the purin bases are converted into uric acid.

The destruction seems to take place chiefly in the liver, kidney, and muscles, extracts of these organs being capable of destroying uric acid *in vitro*; but little or no uric acid is destroyed in the lungs, spleen, and blood. In different species of animals the amount of destruction in the liver and kidney varies, in carnivora the liver being most active, as shown by Burian and Schur, who found that in nephrectomized dogs no uric acid appears in the blood, but on excluding the liver from the circulation, uric acid at once appears. In herbivora the kidneys seem to be more actively uricolytic. This difference perhaps depends upon

the large amount of purin bodies brought to the liver from the animal food of the carnivora. In man the kidney is also most active, although if the bulk of the organs be taken into account, in man the muscles destroy most uric acid, next the kidneys, and then the liver (Croftan). The destruction seems to be accomplished by specific *uricolytic enzymes*, which are of the nature of oxidizing enzymes, although it may be that some other enzymes must first split the uric-acid molecule to prepare it for oxidation.

THE OCCURRENCE OF URIC ACID IN THE BLOOD, TISSUES, AND URINE

As can be seen from the foregoing discussion, the amount of uric acid that appears in the urine depends upon a number of factors, which may be enumerated as follows: (1) The amount of purin bodies taken in the food, upon which, chiefly, depends the amount of exogenous uric acid. (2) The amount of destruction of tissue nucleoproteids. (3) The amount of purin bases formed in the muscle tissue. (4) The amount of conversion of purin bases into uric acid. (5) The amount of destruction of uric acid occurring in the body. (6) Possibly upon the capacity of the tissues to synthesize uric acid; and in case such power to synthesize uric acid exists, upon the presence of the precursors of uric acid in the body. (7) The retention of uric acid in the blood and tissues. (8) The power of the kidneys to excrete uric acid.

If we also take into account the fact that the solubility of uric acid in the urine depends chiefly upon the amount of neutral phosphates present in the urine, and also upon the temperature, reaction, and concentration of the urine, it becomes apparent how totally devoid of significance is the presence of crystals of uric acid and urates in the urine, and how fallacious is any theorization based upon the excretion of considerable quantities of uric acid when all the above-mentioned factors, especially the diet, are not controlled and taken into consideration. Yet on just such an inadequate basis has been constructed an enormous amount of theorization as to "uric-acid diathesis," "uric-acid intoxication," "lithemia," etc., until it has come to be popularly believed that a large share of the minor ailments of humanity, and in particular all non-infectious diseases of the joints and muscles, are dependent upon the presence of excessive quantities of uric acid or urates in the blood. But it may be safely stated that at the present time there exists no good evidence which makes it probable that uric acid is responsible

for any pathological conditions whatever, except uric-acid calculi, "uric-acid infarcts" in the kidneys, and certain manifestations of gout. Uric acid is possessed of but a very slight degree of toxicity, and the body is able to destroy it in such large measure that an actual intoxication with uric acid probably never occurs.

The amount present in the urine may be very considerably increased by eating food rich in purins, of which sweet-breads, liver, and kidney are the best examples; and also coffee with its caffein (trimethyl purin¹). Large quantities of meat will also increase the uric acid, because of the free purins contained in muscle. However, the amount of uric acid in the blood is not correspondingly raised, this being regulated by the destructive and binding function of the tissues, and by excretion through the kidneys. Whenever much destruction of the nucleoproteids of the tissues is occurring in the body, the elimination of endogenous uric acid becomes abnormally raised, the best examples being the resolution of pneumonic exudates, and leukemia, especially leukemia under *x*-ray treatment (*q. v.*). In neither of these conditions, however, can any symptoms or tissue changes be referred to the excessive uric acid. It is quite possible that the power of the body to oxidize uric acid may be decreased under certain conditions; thus, alcohol is found to cause a decided increase in uric-acid elimination, particularly after purin-rich foods have been taken (Chittenden), and this effect is ascribed to lessened uric-acid destruction. However, we have no evidence that, except possibly in gout, this decreased destruction of uric acid under the influence of alcohol causes harm. It has been shown that severe organic lesions involving the liver (phosphorus poisoning) do not cause a marked decrease in the power of oxidizing uric acid.

GOUT

After adjusting the many contradictory statements of earlier investigators, the present status of our conception of uric-acid metabolism in gout may be briefly summarized as follows: The excretion of uric acid in patients with *chronic gout*, when kept upon a definite diet, does not differ from the excretion of normal individuals on the same diet, except in cachectic arthritics, with whom the elimination of uric acid is small. Normally the elimination of uric acid varies within rather wide limits, even on

¹ Concerning the effect of diet upon purin excretion see Taylor, Amer. Jour. Med. Sci., 1899 (118), 141.

a constant diet, and the variations in chronic gout fall within the same limits. There is always an increased amount of uric acid in the blood in gout, but no constant increase can be noted preceding the attack (Magnus-Levy). In the intervals between the attacks of *acute gout* the elimination of uric acid remains within the normal limits; however, for a period of one to three days before each acute attack the amount of uric acid is usually decreased considerably. With the onset of the attack the amount of uric acid excreted becomes increased, and for a few days remains above the average, then subsides to about the normal. Of these two features, the increased output of uric acid during the attack seems to be more constant than the reduced output preceding it.

As yet, however, we have no definite information either as to the cause of this behavior of the uric acid during the paroxysms of acute gout, or as to its part in causing the paroxysm. However, in view of the fact that monosodium urate is found in the joints during the attacks, it seems most probable that for some as yet unknown reason there occurs a precipitation or anchoring of the urates in the tissues, which is associated with the attacks of pain and swelling. We do not know, however, that it is the deposition of urates that causes the attacks. Indeed, the fact that uric-acid retention precedes the attack, rather than accompanies it, seems to suggest that it is the absorption of the urate rather than its deposition in the joints that is responsible for the local disturbances. It is also possible that during the period of retention the uric acid is held in the blood in some form that cannot be eliminated by the kidney, and that its deposition in the joints in an absorbable form occurs simultaneously with the attack.

It should be mentioned in addition that it is not the uric-acid metabolism alone that is altered in gout. Irregular periods of nitrogen retention and nitrogen loss are quite constant features. The cause of this variability, and the form in which the nitrogen is retained, are quite unknown, although there is some evidence that the retained nitrogen is in the form of purin bodies (Vogt).¹ Most of the excessive loss occurs during the acute attacks,¹ and the retention of nitrogen between attacks may be partly to repair the loss; against this, however, is the fact that there is not sufficient gain in weight to account for all of the nitrogen retention. The statements in regard to phosphoric acid elimination, which depends largely on decomposition of

¹ Brugsch, Zeit. exp. Path. u. Ther., 1906 (2), 619.

nucleins, are contradictory, but it seems probable that it shows no characteristic alterations in gout.

It may be seen from the foregoing discussion that we neither understand fully the intricacies of metabolism in gout, nor know whether uric acid is responsible for either the acute painful attacks or for the anatomical alterations in the kidneys, heart, and bloodvessels. It is very possible that some entirely different product of metabolism than uric acid is responsible for most of the changes and symptoms of gout¹—indeed, this would seem to be the case were it not for the great frequency of the deposition of monosodium urate in the joints and cartilages, both during the acute attacks and in chronic gout. This indicates that there is surely something abnormal in the conditions of uric-acid solution and circulation. Why the urate is precipitated in these definite places is another of the many unsolved problems of gout. That it is due to an excess of uric acid in the blood seems to have been excluded, and there is no good evidence that the precipitation depends upon a decreased alkalinity of the blood—two ideas once in vogue. The local nature of the deposition indicates that it must depend upon local changes; but the hypothesis that there occur first degenerative changes in the tissues which determine the precipitation of the urate, seems to have been disproved by the demonstration that the deposition of the urates precedes the necrosis. The histology of urate deposits, both experimental and gouty, has been carefully studied by Freudweiler,² His,³ Krause,⁴ and Rosenbach.⁵

Their results all indicate that uric acid and urates excite some slight inflammatory reaction, cause a slight local necrosis, and seem to act as a weak tissue poison (His). However, they may be deposited without causing necrosis (Rosenbach). Possibly part of the material observed in areas of urate deposition, and generally considered as necrotic tissue, merely represents the framework of the crystalline deposit (Krause). When experimentally injected, the urates are absorbed slowly by phagocytic leucocytes and giant-cells. Why the gouty tophi can be deposited in the chronic process and cause no pain or inflammation, while in acute gout deposition of urates seems to cause such marked symptoms, is also an unanswered question; unless we accept the explanation that the slower

¹ In swine a "guanin gout" occurs; see Schittenhelm and Bendix, *Zeit. physiol. Chem.*, 1906 (48), 140.

² *Deut. Arch. klin. Med.*, 1899 (63), 266.

³ *Ibid.*, 1900 (67), 81.

⁴ *Zeit. klin. Med.*, 1903 (50), 136.

⁵ *Virchow's Arch.*, 1905 (179), 359.

rate of deposition and the lack of dissolved urates account for the absence of symptoms with the tophi.¹

That urates may cause necrosis of the tissues has been definitely established, and this may lead to connective-tissue formation and contraction.² But the actual increase of uric acid in the blood and tissues in gout is so slight that we are not warranted in saying that the usual tendency to sclerosis in all the organs in gout is due to the action of uric acid, rather than to some other unknown agent or agents. Excess of uric acid in the blood is by no means pathognomonic of gout, for it has been observed also in nephritis, in diseases with corpuscle destruction, and after taking purin-rich food. Furthermore, it is quite possible that the precursors of uric acid, the purin bases, are responsible for more harm than the uric acid itself. Thus, administration of adenin to dogs and rabbits will produce degenerative changes in the kidneys, associated with the deposition of substances resembling uric acid and urates in the renal tissue; and Mandel³ states that purin bases may cause fever, independent of infection. In this connection it may be mentioned that many have looked upon renal alterations, leading to failure of excretion of uric acid, as the primary cause of gout; but the evidence in favor of this is faulty, because frequently renal changes are slight or entirely absent in gout, whereas marked nephritis of all forms may exist without the coëxistence of gout.

URIC-ACID INFARCTS

Uric-acid infarcts, as the deposits of urates and uric acid observed in the kidneys of at least half of all children dying within the first two weeks of life are called, give evidence of the slightness of the toxic effects of these substances upon the tissues. Usually little or no change occurs in the renal tubules as a result of these depositions, except such as can be attributed to their mechanical effect.⁴ The reason for the formation of these

¹ Almagia (Hofmeister's Beitr., 1905 (7), 466) has found that joint cartilage placed in urate solutions becomes filled with crystals, which infiltration does not occur with cartilage of any other origin, or with tendons.

² Because the gouty tophi do not suppurate, even when ulcerated through the skin, it has been suggested that the urates have antiseptic properties. Bendix (Zeit. klin. Med., 1902 (44), 165), however, could not demonstrate such antiseptic properties experimentally.

³ Amer. Jour. Physiol., 1904 (10), 452.

⁴ I have recently observed a case of fatal *hematuria neonatorum*, associated with most extensive hemorrhagic infarction of both kidneys. In the bloody urine *B. coli* was found in large numbers. From the anatomical findings and history it seemed quite possible that the injury of the kidneys by uric-acid infarcts might have determined the localization of the bacteria in these organs, with resulting hemorrhages.

infarcts is not at all understood. Spiegelberg¹ found it possible to cause them experimentally in young dogs, in which they do not occur naturally, by injection of 0.25 gram of uric acid per kilo. He was unable to explain why this deposition should occur in young animals but not in old, for he could not find evidence of lessened oxidative power on the part of young animals, and the solvent power of infants' urine was found equal to or greater than that of adults. Other authors, however, have found a lower oxidative power in young animals, and, as favoring the idea that infants have less power to oxidize uric acid than adults, is the fact that allantoin has been found in their urine. Possibly the uric-acid infarcts of infants are the result of the great destruction of nucleoproteids that results from the change of the nucleated fetal red corpuscles to the non-nucleated adult form. McCrudden considers the high concentration of infants' urine an important factor. Minkowski² observed that administration of adenin to dogs led to a deposition of uric acid or some similar substance in the kidneys. Schittenhelm³ found the same deposits in the kidneys of rabbits fed adenin, but not when they were fed guanin. According to Nicolaier,⁴ the crystals thus deposited are not uric acid or urates, but 6-amino-2-8-dioxypurin, derived from the adenin (6-amino-purin) by direct but incomplete oxidation. He could not find this substance in either human urine or in a uric-acid calculus. These experimental infarctions are undoubtedly related to the human form, and indicate that the latter depend upon the presence of an excessive amount of unoxidized uric acid in the body.

¹ Arch. exp. Path. u. Pharm., 1898 (41), 428.

² Arch. exp. Path. u. Pharm., 1898 (41), 375.

³ *Ibid.*, 1902 (47), 432.

⁴ Zeit. klin. Med., 1902 (45), 359.

CHAPTER XXII

DIABETES

As with gout, diabetes has been the subject of such an enormous amount of discussion and experimentation that it is impossible in this place to attempt to review the entire history and literature of the subject, which has already been thoroughly done by a number of physiological chemists and clinicians in the places cited below.¹ In this chapter will be given as briefly as possible merely an epitome of the views now held by the best authorities concerning diabetes, and the problems of carbohydrate metabolism in as far as they relate to diabetes.

Diabetes is usually distinguished from transient forms of glycosuria, although it is well understood that no sharp line between many conditions of transient glycosuria and chronic glycosuria, or diabetes, can always be drawn. In diabetes the sugar present in the urine is predominantly dextrose, small quantities of levulose and other sugars frequently accompanying it. There exist cases, however, in which the urine contains for a long period of time other sugars, particularly levulose and pentose, but these cases are not associated with the profound systemic disturbances of diabetes, presumably because these sugars are not of such great importance for the nutrition of the body as is dextrose.

Glycosuria may be produced by many different causes, which may be grouped under the following heads: (1) alimentary; (2) nervous; (3) drugs and other chemicals; (4) pancreatic.

1. ALIMENTARY GLYCOSURIA

Under ordinary conditions the sugars taken with the food, or formed from the carbohydrates of the food, are in large part converted into glycogen, and temporarily stored in this form. The arterial blood contains quite constantly a small amount of

¹ Pflüger, Pflüger's Arch., 1903 (96), 1; v. Noorden, "Die Zuckerkrankheit und ihre Behandlung"; also translation of his Herter lectures, entitled, "Diabetes Mellitus," New York, 1905, (without bibliography); Macleod, pp. 312-386, in "Recent Advances in Physiology and Biochemistry," London, 1906; Abderhalden, "Lehrbuch der physiologischen Chemie," Berlin, 1906, pp. 13-108. References will generally be cited only when not contained in the above reviews.

sugar, which varies little under normal conditions from 0.1 per cent., or one part in a thousand. By converting the sugar brought to it in the portal blood into glycogen, the liver maintains the proportion of sugar in the blood constant at this small figure. The power of the liver to store up glycogen is not unlimited, however, and hence if too large quantities of sugar are absorbed into the portal vein in a short space of time, not all of it is converted into glycogen in its passage through the liver, and consequently the systemic blood becomes loaded with more than the normal amount of sugar. As soon as this happens the urine begins to contain sugar, for, while the kidney does not excrete more than the most minute traces of sugar from the normal blood, yet any excessive sugar is eliminated at once. The explanation of this will be discussed later.

Since the storage of sugar as glycogen is performed chiefly by the liver, this fact has been used clinically as a test of the functional capacity of the liver. A normal individual can take from 150 to 200 grams of glucose at one time without glycosuria resulting; therefore, if after administration of somewhat smaller quantities, say 100 grams, sugar appears in the urine, we have evidence that the liver is functionally incapacitated. Thus, in cirrhosis of the liver glycosuria often follows the taking of 100 grams or less of glucose. This "assimilation limit" varies under normal conditions for different carbohydrates.¹ Unlimited quantities of starch may be taken, because its rate of conversion into sugar is slow enough to prevent an overwhelming of the portal blood with glucose. Of the sugars, glucose has the highest assimilation limit (150–200 grams); but that of levulose is about as high² (140–160 grams), and the sugar eliminated in the urine when levulose is taken is chiefly glucose mixed with some levulose (v. Noorden). Cane-sugar has about the same assimilation limit as glucose, but lactose (milk-sugar) has a limit of 120 grams or less. With the two disaccharides just named, any excess that is absorbed unchanged from the intestine into the blood reappears in the urine, for they cannot be utilized by the liver or other tissues; maltose alone of the disaccharides can be split in the blood, where a specific ferment, maltase, is normally present. Pentoses can be assimilated to but a very moderate degree, for when even so little as 30 to 50 grams is taken by mouth, a large amount may reappear in the urine.

¹ See Blumenthal, Hofmeister's Beitr., 1905 (6), 329.

² 1.9 gm. per kilo in man, according to De Rossi (Riforma Med., 1904 (20), 729).

Pentosuria.—Pentoses are the chief carbohydrate groups of the nucleoproteids, but they are also present in many vegetables and fruits. Some persons seem to lack in some respects the power of utilizing pentoses, and, therefore, exhibit a chronic pentosuria,¹ while at the same time they can utilize other sugars without difficulty.² They eliminate pentose in the urine even when there is none in the food, but seem able to utilize pentose taken in the food nearly as well as normal individuals. According to Neuberg, the pentose found in the urine is not the same as that of the nucleoproteids, and is possibly derived from the hexoses.³ This condition is not associated with constitutional disturbances, and must be considered as an anomaly in metabolism similar to cystinuria and alkaptonuria, especially since it may occur as a family disease. True diabetes may also, in certain cases, be looked upon as an hereditary metabolic disorder, in view of the frequently observed occurrence of the disease as a family peculiarity. Lorand⁴ has observed that the children of diabetics may show a defective power of sugar assimilation.

Levulosuria, in which levulose is eliminated in the urine on a diet containing moderate amounts of levulose, is a rare condition. Neubauer⁵ has collected reports of five cases in which levulose alone was present in the urine, without dextrose being present. As levulose is apparently converted into glycogen, which then breaks down into glucose, the failure of assimilation of levulose in these patients would seem to be due to a failure of the conversion of levulose into glycogen. Neubauer observed in his case that a definite proportion (15–17 per cent.) of the levulose given by mouth was excreted in the urine, and suggests as an alternative hypothesis that a certain proportion of the levulose of the food is directly oxidized without formation of glycogen, and that failure of this oxidation may be the cause of levulosuria. Mixed levulosuria and glycosuria is relatively frequent, and in some cases, at least, the levulose in the urine seems to have been derived from the glucose in the body and not from the levulose of the food.

Lactosuria, or excretion of milk-sugar in the urine, has rarely been observed as a form of alimentary glycosuria, but is frequently observed in connection with formation of milk in the mammary gland both before and after parturition.⁶ After resection of the mammary glands lactosuria does not occur (Moore and Parker⁷).

Alimentary glycosuria, following administration of small quantities of glucose, does not necessarily mean that the function of the liver is primarily decreased; in some cases the

¹ Literature reviewed by Neuberg, *Ergebnisse der Physiol.*, 1904 (3, Abt. 1), 373; Wohlgenuth, *Biochem. Centralbl.*, 1903 (1), 533. More recent articles by Jolles, *Cent. f. inn. Med.*, 1905 (26), 1049; Adler, *Pflüger's Arch.*, 1905 (110), 625; Tintemann, *Zeit. klin. Med.*, 1905 (58), 190; Erben, *Prag. med. Woch.*, 1906 (31), 301; Blum, *Zeit. klin. Med.*, 1906 (59), 244; Janeway, *Amer. Jour. Med. Sci.*, 1906 (132), 423.

² Pentosuria may be associated, or occur alternately, with glycosuria; see Kaplan, *New York Med. Jour.*, 1906 (84), 233.

³ Many unfermented fruit-juices—*e. g.*, apple-juice—contain much pentose, which may cause alimentary pentosuria when taken in large amounts (v. Jaksch, *Cent. f. inn. Med.*, 1906 (27), 145).

⁴ *Practitioner*, 1903 (71), 522.

⁵ *Münch. med. Woch.*, 1905 (52), 1525 (full literature).

⁶ Full review by Porcher, "De la Lactosuria," Paris, 1906.

⁷ *Amer. Jour. Physiol.*, 1900 (4), 239.

defect may lie in the nervous system, in some cases in the pancreas, the relation of which organs to glycosuria will be considered later; but in any case of alimentary glycosuria the difficulty lies, either primarily or secondarily, with the liver, which, for one reason or another, cannot convert all the glucose into glycogen. If the liver is primarily affected, it is usually found that assimilation of levulose is more affected than assimilation of glucose, and hence levulose is more useful in determining "hepatic insufficiency" than is glucose.

Why the kidney should retain the amount of sugar present normally in the blood, yet excrete that which is in excess, is an unsettled question. What seems to be the most simple explanation is that the normal 0.1 per cent. of sugar in the blood does not exist free, but is combined—partly with lecithin as *jecorin*, partly with proteids. It is certain that at least part of the sugar is so combined, but we do not know how much. If it is practically all combined, as many believe, we can readily understand how the sugar combined with large colloidal molecules could be retained by the glomerular membranes, while the excessive uncombined sugar diffused through into the urine. However, recent work by Asher and Rosenfeld¹ throws considerable doubt on the existence of blood-sugar in a non-diffusible combination.

2. NERVOUS GLYCOSURIA

The classical example of glycosuria due to nervous impulses is that discovered by Claude Bernard, who found that a minute puncture of the floor of the fourth ventricle, between the roots of origin of the eighth and tenth pairs of nerves, causes glycosuria. This glycosuria begins in about an hour after the puncture (*piqûre*) is made, and *lasts only as long as glycogen remains in the liver*, for this form of diabetes depends upon the rapid conversion of the glycogen of the liver into sugar. Because of this excessive liberation of sugar, the blood contains more than the normal amount (*hyperglycemia*), and the excess escapes through the kidneys. If the animal has been starved and exercised, so that the glycogen in the liver is reduced to a minimum, no glycosuria is produced. All of the excess of sugar comes from the liver, for if the hepatic vessels are first ligated, no glycosuria results from puncture.

This "*diabetic*" or "*glycogenic center*" seems to exist in all varieties of animals, and in man chronic glycosuria has been observed as a result of tumors or other lesions involving this

¹ Cent. f. Physiol., 1905 (19), 449.

part of the brain. Glycosuria results from irritation, not from destruction of the center. Undoubtedly the glycogenic center has an important function in regulating glycogen deposition in the liver. That it exercises this function through nervous impulses passing directly from the brain to the liver, has been conclusively shown by experimentally severing various parts of the nervous system in animals whose diabetic centers have been punctured. If the vagus is cut, stimulation of its central end causes glycosuria, indicating that the afferent impulses travel through this nerve, and glycosuria has been observed in persons with tumors pressing upon the vagus. The efferent impulses pass in the spinal cord from the glycogenic center to the upper thoracic spinal roots, and by the *rami communicantes* into the lower cervical and upper thoracic sympathetic ganglia; thence by the splanchnic nerves to the liver. How the nervous impulses cause the discharge of sugar is unknown, but possibly it is by some direct stimulation of the cells, as with other secretory impulses. Against this, however, is the fact that atropin, which paralyzes all true secretory nerve-endings, does not prevent glycosuria from piqure. Bernard thought that the sugar production was increased merely by vasodilation, and in favor of this view is the fact that a fall of blood pressure decreases or prevents the glycosuria.

In man, glycosuria may result from injuries to the head, presumably because of irritation or stimulation of the glycogenic center. In many nervous diseases more or less transient glycosuria may occur, and an existing glycosuria may be augmented by nervous stimuli. Administration of thyroid extract, and exophthalmic goiter, may cause glycosuria, presumably from nervous stimulation. Undoubtedly, many of the drugs that cause glycosuria do so through their stimulation of this center.

3. DRUG GLYCOSURIA

A. Phlorhizin "Diabetes."¹—This is by far the best known and most studied instance of glycosuria produced by the action of drugs, and offers many points of particular interest. Phlorhizin is a glucoside, obtained from the bark of apple and pear trees, which may be split into dextrose and *phloretin*, the latter causing the characteristic glycosuric effects. When given by mouth or subcutaneously (the usual dose for dogs is 1 gram per kilo, by mouth, and 0.3 to 0.5 gram subcutaneously), it

¹ See Lusk, *Zeit. f. Biol.*, 1901 (42), 31; Cramer, *Ergebnisse der Physiol.*, 1902 (1), 877.

causes a transient but marked glycosuria; as much as 19 per cent. of sugar may be present in the urine of well-fed animals, and from 0.3 to 2.5 per cent. if the animals are starving. The way in which phlorhizin causes glycosuria is not fully determined. It has been generally considered that it acts directly upon the kidneys, so that they excrete sugar from the blood until it contains much less sugar than normal. That the drug acts directly upon the kidney is apparently proved by Zuntz's experiment, which consisted of injecting phlorhizin into one renal artery, with the result that sugar appeared in the urine from this kidney at once, and considerably later in the urine from the other kidney. It would also seem that the excretion of sugar does not depend upon a renal lesion that causes the glomerules to "leak" sugar, but rather upon a direct secretory activity of the kidney, since any disease or injury of the renal tissue diminishes or prevents the glycosuria following phlorhizin administration, although phlorhizin itself causes necrotic changes in the renal epithelium.

However, these views have been vigorously opposed. Pavy claims that the blood in phlorhizin diabetes does not contain less sugar than normal,—indeed, he found that it might contain more,—but his results are in contradiction to those of many others, who have found a decreased amount of sugar in the blood (*hypoglycemia*) as a characteristic feature of phlorhizin diabetes. Pflüger questions Zuntz's results on the ground that the sugar observed in the urine might have come from the phlorhizin itself, through splitting, and questions the justification of considering phlorhizin glycosuria as a special, peculiar form of glycosuria, differing from all other forms, which depend upon a hyperglycemia.

After repeated doses of phlorhizin, the glycogen may disappear to a large extent from the liver, and also from the muscles, but the reduction is by no means so marked as in some other forms of glycosuria. Apparently, phlorhizin does not act upon the glycogenic function of the organs, but simply causes a draining away of sugar, to replace which the glycogen is converted into sugar. Corroborating this view is the fact that if the kidneys are tied off, administration of phlorhizin does not cause a rise in the amount of sugar in the blood.

If an animal to which phlorhizin is being repeatedly given is starved, the elimination of sugar does not cease, but continues at a low level, while at the same time the elimination of nitrogen is increased until a maximum constant ratio of nitrogen to sugar is established, with the proportion of dextrose to nitrogen

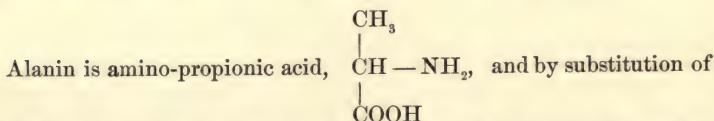
(the "D:N ratio") staying at 3.75:1 (Lusk). In pancreatectomized dogs the D:N ratio is about 2.8:1 during starvation (Minkowski). This and other facts seem to indicate that under these conditions sugar is formed from the body proteids. This brings forward the long-contested question as to the possibility of:

THE FORMATION OF SUGAR FROM PROTEIDS¹

In favor of this source of sugar has long been known the fact that diabetics, when kept for a long period on carbohydrate-poor diet, excrete sugar in quantities out of all proportion to the amount of carbohydrate in the food. At the same time the amount of nitrogenous elimination will be found to be excessive, supporting the idea that the sugar of the urine may have been derived from the breaking down of proteids. If the patient is kept for some time on a diet both free from carbohydrates and poor in proteids, it will be found that the addition of proteid to the diet causes at once an increased elimination of sugar in the urine. Nor is all this sugar derived from the carbohydrate groups of the proteid, for, firstly, it may greatly exceed the amount of carbohydrate groups contained in the proteid; and, secondly, the amount of sugar escaping in the urine does not vary according to the amount of carbohydrate in the proteid of the food: *e. g.*, casein is free from carbohydrate groups, but it may cause more increase in glycosuria than does egg-albumen, which is rich in carbohydrate groups.²

It has been demonstrated experimentally that carbohydrates can be formed from the amino-acids of the proteid molecule. If alanin is given to diabetics, it will be found to give rise to almost equivalent quantities of sugar; with normal persons or animals this conversion of alanin into sugar does not seem to occur. Similar, but somewhat less conclusive evidence has been obtained that glycocoll and leucin³ may also be changed into sugar. Feeding of alanin to starved rabbits may cause an increase in the glycogen in the liver; and starved animals poisoned with phlorhizin excrete more sugar when alanin is given them.⁴

Presumably, therefore, amino-acids liberated from the proteids during their metabolic decomposition can give rise to carbohydrates. The steps by which alanin might be changed into dextrose are as follows:



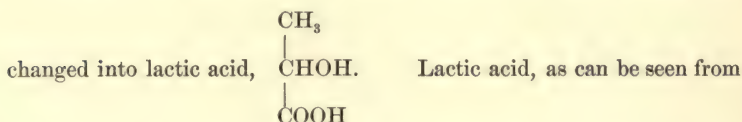
an OH group for the NH₂ group, a process that may readily occur in the body through the action of deamidizing enzymes (amidase), it is

¹ Literature by Langstein, *Ergebnisse der Physiol.*, 1902 (Bd. 1, Abt. 1), 63; 1904 (Bd. 3, Abt. 1), 453; Therman, *Skand. Arch. f. Physiol.*, 1905 (17), 1.

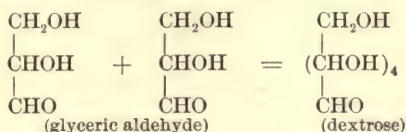
² See Therman, *loc. cit.*

³ Mohr (*Zeit. exp. Path. u. Ther.*, 1906 (2), 463) has noted the elimination of leucin fed to diabetics, in the form of a polypeptid.

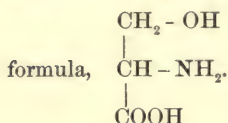
⁴ See Almagia and Embden, *Hofmeister's Beitr.*, 1905 (7), 298.



the formulæ, is closely related to glyceric aldehyde, which in turn may readily be condensed into dextrose, as follows :



Serin, oxy-amino-propionic acid, also a constituent of the proteid molecule, is even more closely related to dextrose, as shown by its



Extreme difficulties exist in such experimental work, because of the numerous possible sources of error which are introduced by the following conditions : (1) More or less glycogen is retained in the tissues during starvation ; (2) the proteids of the foods contain preformed carbohydrate radicals ; (3) carbohydrates may be formed from fats ; (4) proteids and amino-acids may be oxidized in place of carbohydrates, which thus escape destruction and cause increased glycosuria. This has made most of the experimental evidence on this question of uncertain value ; consequently, while we find, in a recent review by v. Noorden, the origin of sugar from the proteid molecule treated as an established fact, at the same time Macleod, in his review of the literature,¹ states that "there is no unequivocal evidence, so far" that glycogen formation can result from feeding with proteids that contain no carbohydrate group. However, Macleod also states that by the indirect method "the evidence undoubtedly points to sugar formation from all proteids."

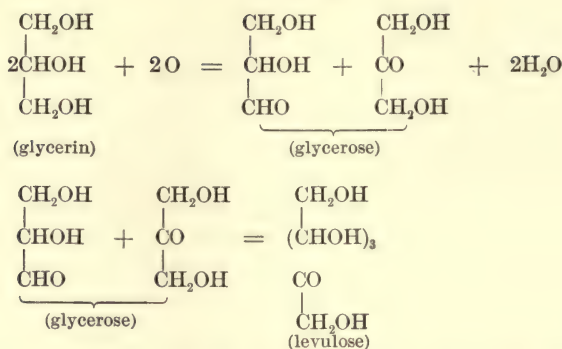
FORMATION OF SUGAR FROM FATS

In starving animals with glycosuria, or in diabetics on a restricted diet, the amount of sugar eliminated often seems to be greater than can be accounted for by destruction of the proteid of the food and tissues, as measured by the nitrogen excretion. It would, therefore, seem probable that sugar may, in these conditions, be formed from the fats.² There is no *a priori* reason why this should not occur, but the proof that it does occur seems to be scanty.

Glycerin might readily form sugar, as follows :

¹ "Recent Advances in Physiology and Biochemistry," 1906.

² Not in phlorhizin diabetes, according to Lusk (*loc. cit.*).



Administration of glycerin has been found to increase the amount of sugar in the urine in glycosuria. No direct evidence that the higher fatty acids form carbohydrates has yet been brought forward, but v. Noorden believes that this must occur, since in some cases of diabetes he has found more sugar in the urine than could be accounted for by the other known sources, including glycerin.

B. Other Substances Causing Glycosuria.—A large number of substances may cause a greater or less amount of glycosuria, when taken by mouth. Presumably they act in different ways. Some of them may stimulate the glycogenic center; this seems to be the cause of the glycosuria following injections of slightly hypertonic solutions of sodium salts (and which can be checked by calcium solutions), for when the splanchnic nerves are cut glycosuria ceases (Martin Fischer¹). Strychnine, phosphorus, arsenic, uranium salts, bichloride of mercury, carbon monoxid, amyl nitrite, curare, chloral, nitrobenzol, chloroform, acetone, ether, etc.,² may all cause glycosuria, but for most of them the point of attack has not been determined. Probably some, like salt solution, and also morphine, attack chiefly the glycogenic center. Others, among which may be included alcohol and the toxins of acute infectious diseases, seem to injure the pancreas particularly. Caffein and diuretin both may cause glycosuria, and, since the chief characteristic of each drug is to cause polyuria, it has been thought that they act primarily upon the kidney, like phlorhizin, but this has not been finally established. Uranium salts are also supposed to cause glycosuria through direct action upon the kidney cells.

¹ Underhill and Closson (Amer. Jour. Physiol., 1906 (15), 321) consider the glycosuria due to increased renal permeability, because a hypoglycemia is observed.

² Literature given by Abderhalden (*loc. cit.*).

Adrenalin glycosuria, which follows administration of active preparations of the adrenal by any route, has been discussed elsewhere (see "Adrenal," page 501). The most marked effects follow intraperitoneal injection, apparently because of direct action upon the pancreas, for minute quantities painted upon the surface of the pancreas cause a prompt glycosuria (Herter and Wakemann¹). Underhill² has found that piperidine produces similar effects, but also causes glycosuria equally well if painted upon the spleen, the effect being prevented by administration of oxygen. He suggests that piperidine, potassium cyanide, ether, chloroform, morphine, strychnine, curare, and many other similar substances owe their effect to an action upon the respiratory center, causing dyspnea and consequent diminution of oxidation of carbohydrate material. Adrenalin glycosuria, however, is not prevented by administering oxygen; therefore, it must be considered as essentially different from the glycosuria caused by the above-mentioned chemicals.³

4. PANCREATIC GLYCOSURIA

This form of glycosuria is of the greatest interest, not only because it seems to be most closely related to human diabetes, but also because it opens up for consideration some of the long obscure points concerning the internal secretion of the pancreas and carbohydrate metabolism. Since the experiments of v. Mering and Minkowski in 1889,⁴ we have known that extirpation of the pancreas results in severe glycosuria, and that this depends upon the lack of some internal secretion of the pancreas, and not upon absence of the pancreatic juice that escapes into the intestine. This last point was conclusively shown by the fact that retention of a small portion of the pancreas—from 10 to 20 per cent. of its original bulk—prevents the development of glycosuria, even when the retained portion has been transplanted to another part of the body. The glycosuria appears from three to five hours after the operation; at first profound, it decreases in amount as the glycogen is lost from the liver, but never disappears, even

¹ Iwanoff (Cent. f. Physiol, 1906 (19), 891) has found that adrenalin increases the rate of discharge of sugar from the isolated, glycogen-rich liver, through which salt solution is being transfused.

² Jour. Biol. Chem., 1905 (1), 113.

³ Glaessner (Wien. klin. Woch., 1906 (19), No. 30) has observed transient glycosuria following severe over-cooling; *e. g.*, attempted drowning. The glycosuria is probably due to defective oxidation.

⁴ "Diabetes Mellitus nach Pankreasextirpation," Leipzig, 1889; also Arch. exp. Path. u. Pharm., 1890 (26), 371.

if the animal is starved. Feeding of carbohydrates increases it greatly, and the greater part of the sugar administered may appear in the urine. Pancreatectomized dogs live at most two to three weeks, death being due not so much to the disturbance of metabolism as to infection of the operation wounds, which heal poorly because of the high sugar content of the blood (Pflüger).

While the amount of glycogen in the liver and muscles decreases greatly, the amount of sugar in the blood is increased. Instead of the normal 1 part per thousand, as much as 7 to 10 parts of sugar may be present per thousand parts of blood, and the glycosuria is, as in the case of nervous glycosuria, dependent upon hyperglycemia and consequent elimination of the excessive sugar by the kidneys. Since it has been proved that absence of the pancreatic juice is not responsible for the glycosuria, the only remaining explanation of this hyperglycemia is that it is the result of the loss of some internal secretion, or the absence of some direct action of the pancreas itself upon the blood passing through it. The following explanations suggest themselves :

- (1) The pancreas may directly destroy sugar coming to it in the blood.
- (2) It may secrete an enzyme that destroys sugar in the blood.
- (3) It may neutralize or destroy some toxic substance that interferes with sugar metabolism.
- (4) It may secrete some substance that is itself necessary for proper sugar metabolism in the liver and other tissues of the body.

As to the first possibility, it can only be said that we have no evidence whatever that the pancreas is the site of any considerable active sugar destruction. Repeated investigations have failed to show that the pancreas has any marked powers of glycolysis, or contains any particularly active glycolytic enzyme as compared with other organs. That the chief function of the pancreas in carbohydrate metabolism consists of furnishing a glycolytic enzyme to the blood has been completely disproved. The blood does exhibit some glycolytic power, but this is far too slight to account for the daily destruction of several hundred grams of sugar, and, furthermore, there is every reason to believe that this destruction takes place in the tissues, and not in the blood. If the pancreas is removed, the glycolytic power of the blood is not decreased, showing that it is not derived from the pancreas, and the blood of the pancreatic vein is no

more actively glycolytic than the blood of the general circulation.

While Tuckett¹ and some others have claimed that the function of the pancreas is to neutralize toxic substances entering the blood from the alimentary tract, or formed in metabolism, as yet their results do not seem to have received general acceptance. The demonstration by Minkowski and v. Mering that the blood of pancreatectomized dogs does not contain substances causing glycosuria in normal dogs, seems to still stand as evidence that the glycosuria does not depend upon accumulated toxic substances. Lombroso² disproved the hypothesis that glycosuria after pancreatectomy is due to absorption of toxic substances formed in the intestine because of the defective pancreatic digestion, by the following experiment: The fluid escaping from a pancreatic fistula of one dog was injected into the duodenum of a pancreatectomized dog; although the digestion of the second dog was much improved, the glycosuria was not reduced.

The Internal Secretion of the Pancreas.—There remains, consequently, only the hypothesis that the pancreas secretes some substance that directly or indirectly modifies sugar metabolism, and to determine what this substance might be has been the object of many investigations. Most suggestive of the results obtained are those of O. Cohnheim, who discovered evidence that *the internal secretion of the pancreas has the function of activating an inactive glycolytic enzyme* that is contained in the liver, muscles, and other tissues. Thus, he found that whereas the expressed juice of fresh muscle tissue, and the juice of fresh pancreas tissue, are each alike possessed of but slight glycolytic properties, yet when a small amount of the pancreatic extract is added to the muscle-juice, the mixture is capable of rapidly destroying dextrose. Enough destruction of sugar occurs in these experiments to account fully for the great amount of sugar that is daily destroyed in the human body.³ The products of this glycolysis are carbon dioxide and water, if an abundance of oxygen is present; but in the absence of oxygen, first alcohol, then lactic acid, and later oxybutyric

¹ Jour. of Physiol., 1899 (25), 63.

² Ref. in Biochem. Centr., 1903 (1), 346.

³ Rahel Hirsch, independently of and simultaneously with Cohnheim, observed that glycolysis by liver tissue is increased upon the addition of pancreatic extract. A number of observers, especially Jacoby, Blumenthal, Feinschmidt and Arnheim, claim that the liver, and possibly other organs, contain active glycolytic enzymes, but it may be that these are active only because of the presence in them of the pancreatic secretion brought to them in the blood.

acid, are formed. An important observation, in view of our knowledge of the ability of pancreatectomized animals to utilize levulose, is that the combined pancreas-muscle or pancreas-liver extracts do not destroy levulose.¹

The activating substance may be compared with the *enterokinase* of the intestine, which activates the inert *trypsinogen* of the pancreatic juice; and Cohnheim calls it the "pancreas activator." It acts in very small quantities, is not destroyed by alcohol or by heating to boiling, and excessive quantities prevent activation (similar to Ehrlich's "deviation of complement"). Although numerous objections to Cohnheim's views have been made, yet the main fact that the pancreas produces an activator substance for a glycolytic enzyme contained in other tissues seems to have been safely established.²

Defective Glycogenesis.—This discovery perhaps explains why the power to utilize sugar is reduced, but it does not by any means explain all the features of diabetes following pancreatic injury. One of the most important characteristics of pancreatic diabetes is the almost complete disappearance of glycogen from the liver, and, to a less extent, from the muscles, while at the same time there may be a deposition of glycogen in excessive quantities in other tissues where it does not normally occur abundantly (see "Glycogenic Infiltration," page 363).

This decrease in the glycogen of the liver occurs at a time when the blood contains much more sugar than it does normally, and indicates that the liver has almost entirely lost its normal power of converting all excessive sugar into glycogen. On this account we cannot be completely satisfied with an explanation of pancreatic diabetes that accounts merely for decreased destruction of sugar, as does Cohnheim's pancreas activator. We must also account for the *impaired glycogenesis*. v. Noorden seeks to explain this defective formation of glycogen by the hypothesis that the pancreas furnishes a secretion which either favors the polymerization of sugar into glycogen, or else inhibits the power of the tissues to split up the glycogen that they have formed. Of the two possible errors, excessive destruction and faulty formation of glycogen, he is inclined to favor the latter as the more probable, in view of the well-known fact that pancreatectomized dogs and diabetics can form glycogen out of levulose, when they are unable to form it out of glucose. The added fact that diabetics can frequently utilize levulose, in spite of the fact that the glycogen so formed must later become glucose,

¹ Sehrt, Zeit. klin. Med., 1905 (56), 509.

² See Cohnheim's recent publication, Zeit. physiol. Chem., 1906 (47), 253.

is used as an argument that it is not as sugar that the cells utilize carbohydrates, but as glycogen. In the words of v. Noorden, the natural carbohydrate food of the cell is not glucose, but glycogen.

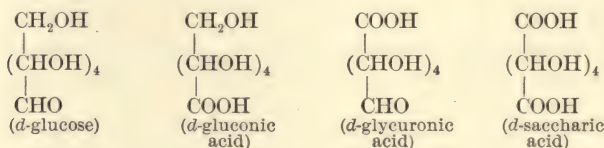
Pflüger takes, as the more probable, the view that it is excessive conversion of glycogen into sugar, rather than defective glycogenesis, that is at fault. The function of the pancreas, on this basis, is the formation of an anti-enzyme that holds in check the diastatic enzyme of the cells. Pavy maintains that after sugar is once built up into glycogen it goes on to form fats and proteids, but does not again break down into sugar under normal conditions. This view, in direct opposition to Bernard's theories of carbohydrate metabolism, is not generally accepted.

Theories as to the Cause of Pancreatic Diabetes.—

With the existing confusion and difference of opinion concerning the importance of defective glycogen formation in diabetes, it is impossible to give an exact or clear idea concerning the rôle of the pancreas in diabetes. Certainly, after pancreas extirpation the liver does not entirely lose its power to form glycogen, for some glycogen may be still present in the liver after the most protracted glycosuria. Neither does defective glycogenesis explain why the blood of starving pancreatectomized animals contains excessive quantities of sugar. This last fact speaks rather in favor of excessive breaking down of the glycogen, analogous to the glycosuria following puncture of the medulla. Possibly, either the glycolytic ferments or the glycogen are normally so combined in the cells that they cannot freely act or be acted upon to form sugar, which combined condition ceases to exist in the absence of the internal secretion of the pancreas. This is, of course, purely hypothetical.

There can be no doubt that the tissue-cells of pancreatectomized animals have lost their power to utilize sugar, leaving out of the question whether this is due to abnormal glycogenesis or to loss of glycolytic power. Feeding of carbohydrate food causes immediately an increase in the amount of sugar excretion, and usually the greater part of the sugar appears again in the urine, showing that it has passed through the body unutilized. Levulose alone seems to be fairly utilized under these conditions. This failure to use the sugar is not due to a decrease in the oxidizing powers of the cells, for other substances seem to be oxidized with quite normal activity. Lactic acid, inosite, mannite, benzol, and many other substances, when given by mouth, are oxidized as in normal animals. The respiratory quotient is affected only as far as the loss of carbohydrates reduces the sum

total of oxidation that is going on. Proteids and fats are oxidized to a large extent, although the appearance of volatile fatty acids in the urine in advanced diabetes may be taken to indicate that the oxidation is not up to normal standards. O. Baumgarten¹ has recently demonstrated that certain partly oxidized carbohydrates can be oxidized completely in the tissues of pancreatectomized dogs. The bodies examined were *d*-gluconic acid, *d*-saccharic acid, mucic acid, glycuronic acid, glycosamin hydrochloride, succinic acid, *d*-tartaric acid, salicylic aldehyde, and vanillin. The relation of some of these bodies to glucose is shown by the following formulæ :



These experiments indicate that the diabetic organism can oxidize sugars after a start has been made on the oxidation, and, as it is unable to oxidize them before this first step has been performed, it is a fair assumption that the difficulty lies with the first attack on the sugar molecule. Baumgarten believes that a "fermentative splitting of the sugar molecule must precede oxidation of the carbohydrates, which splitting is more or less incomplete in diabetes." On the other hand, we have evidence that diabetics are not totally incapable of beginning the oxidation of glucose, for if they receive such substances as are eliminated in the urine combined with glycuronic acid (*e. g.*, camphor, chloral, naphthol, etc.), they eliminate this glycuronic acid compound almost as abundantly as do normal individuals. Indeed, glycuronic acid is frequently eliminated in diabetes, even without the presence of the above-mentioned combining bodies ; and sometimes it may be found in the urine even when, through careful dieting, the excretion of sugar has ceased. As glycuronic acid represents merely the result of the first step of oxidation of glucose, as shown by the formulæ given above, it would seem that the first step of sugar oxidation can be accomplished in diabetes, and that the fault lies rather with the subsequent splitting of the molecule. This view does not harmonize with Baumgarten's experiments, and the disagreement has yet to be explained. In any case, however, experimenters are well agreed that the difficulty in diabetes lies in the

¹ Zeit. exp. Path. u. Ther., 1905 (2), 53.

splitting of the long hexose chain, rather than in the oxidation processes themselves.

HUMAN DIABETES

Diabetes, being characterized by a long-continued glycosuria, we may imagine it to be due to any or all of the causes mentioned in the preceding discussion. As a matter of fact, however, it is in most cases related more closely to pancreatic glycosuria than to the other forms.

Diabetes from an increased permeability of the kidneys, analogous to phlorhizin glycosuria, has not been established as a definite condition in man, although its existence has been suspected and urged more than once. On the other hand, the development in a diabetic of renal lesions, especially chronic interstitial nephritis, may greatly reduce the excretion of sugar in the urine.

True diabetes from *excessive consumption of carbohydrates* is, of course, out of the question, but often in its earliest stages diabetes presents the symptom of glycosuria only when excessive quantities of carbohydrates are taken in the food. Diabetes from overproduction of sugar in the body is also unknown—the abnormally great formation of sugar from proteids (and probably from fats) that occurs in diabetes, is secondary to the loss of sugar and not primary.

True diabetes may, however, result from purely *nervous causes*, exactly analogous to glycosuria following Bernard's puncture of the medulla. This has been observed in a few instances in persons suffering from tumors, hemorrhages, softening, etc., in the vicinity of the diabetic center. There is also much evidence that in diabetes generally the nervous system plays an active part, and nervous shock, depression, etc., are known to exert an unfavorable influence on the course of diabetes. The favorable therapeutic effects obtained in diabetes with opium have been ascribed to the reduction of nervous excitability.¹ It is quite probable that the discharge of glycogen from the liver, which is so marked a feature of diabetes, is brought about by nervous stimuli, similar to those which empty the liver of glycogen when the glycogenic center is irritated. These stimuli presumably arise from the tissues, which are in a condition of sugar starvation because of their inability to utilize sugar, and, therefore, send stimuli calling for more sugar to the glycogen storehouses.

¹ See Meyer, Zeit. exp. Path. u. Ther., 1906 (3), 58.

But, in its cardinal features, *human diabetes most generally resembles pancreatic diabetes*, and in a large proportion of the cases lesions of the pancreas are found present. These are not always marked, however, and they are by no means constant. Opie, in particular, has brought forward evidence that diabetes is frequently associated with lesions of the islands of Langerhans, whereas extensive lesions of the pancreas which do not involve the islands (*e. g.*, sclerosis and atrophy following occlusion of the pancreatic duct) do not cause diabetes. This, of course, suggests that the islands of Langerhans produce the internal secretion of the pancreas that has to do with sugar metabolism. The full evidence on this point will be found in Opie's work on "Diseases of the Pancreas." However, there occur many cases of diabetes in which no anatomical changes whatever can be found in the pancreas, and others in which the lesions in the parenchyma far outweigh those of the Langerhans islands.¹ Still another fact of significance in this connection is that organotherapy, by means of pancreas preparations, has given no favorable results in diabetes, even when the diabetes has been unquestionably of pancreatic origin.²

We must, therefore, recognize the probability that pancreatic disease is not the sole cause of diabetes and that the importance of the islands of Langerhans is not finally established, while at the same time recognizing the fact that failure to detect anatomical changes does not always prove the absence of functional impairment.

The glycosuria, which is the most characteristic, but by no means the sole, important feature of diabetes, is dependent upon hyperglycemia. The amount of sugar in the blood is often as much as three parts per thousand, instead of one part as normally, and may reach 7 to 10 parts; however, the amount of sugar in the urine does not by any means vary directly with the amount in the blood. This hyperglycemia is remarkable in that, at least in the advanced stages of diabetes, it persists even

¹ For the evidence against the view that the islands of Langerhans are the chief factor in pancreatic diabetes see Herxheimer, *Virchow's Arch.*, 1906 (183), 228.

² Moore, Edie, and Abram (*Biochem. Jour.*, 1906 (1), 28 and 446) have obtained a favorable influence in certain cases of diabetes by stimulating the pancreas through administration of *secretin* obtained in duodenal extracts. Secretin is a secretion of the upper part of the small intestine, which has the property of causing a greatly increased flow of pancreatic juice. Presumably it produces its effect in diabetes by increasing the internal secretion of the pancreas. Bainbridge and Baddard (*Biochem. Jour.*, 1906 (1), 429) found the secretin content of the intestinal mucosa greatly decreased in human diabetes, although unable to obtain favorable clinical results by administration of duodenal extracts.

when the patient is receiving no carbohydrate whatever in the food. In the early stages glycosuria may appear only after the taking of carbohydrates, so that it may be entirely suppressed by proper diet. Later, however, as the power to utilize sugar becomes still more impaired, the demand of the tissues for sugar becomes so great that carbohydrates are formed in large amounts from proteids, and perhaps also from fats. At the same time the sugar given by mouth passes unappropriated through the tissues, and the greater part of it, sometimes all, reappears unchanged in the urine. As with experimental pancreatic diabetes, the power to utilize levulose is retained longer than for dextrose, but eventually even the levulose is largely lost, after being partly converted into glycogen.

The power to oxidize substances other than dextrose is at first apparently unimpaired, but later the general oxidative capacity is reduced, and we find large quantities of underoxidized products of metabolism appearing in the urine, especially the "*acetone bodies*." The presence of these organic acids in the blood in large amounts leads to diabetic coma, which is in most respects an *acid intoxication*. (This matter is discussed fully under the topic of "Acid Intoxication," Chap. xviii.) Undoubtedly, other toxic substances also accumulate, and constitute an important, if subordinate, cause of the toxic manifestations of the disease. Failure of oxidation of fats is perhaps responsible for the frequently observed accumulation of large quantities of fat in the blood—*lipemia*. (Discussed under "Fatty Metamorphosis," page 344.) The failure of oxidation of sugar is so marked that it is impossible to cause the glycosuria to be reduced greatly, at least in severe cases, by hard muscular exercise. Thus, a patient who is excreting sugar on a carbohydrate-free diet may climb a mountain or do other severe work which requires normally the burning up of 80 to 100 grams of carbohydrate, and yet continue to excrete nearly or quite as much sugar as he did before. This indicates that all the energy available for the diabetic must come from fats and proteids, because of the inability to utilize sugar under even the most extreme conditions.

In any case, the pathology of human diabetes usually resembles that of diabetes following experimental pancreatectomy in its chief features, and the problems are quite the same as those which have already been discussed in connection with the experimental disease. We are entirely uninformed as to the parts played by defective glycogenesis and by defective oxidation of sugar, independent of a preliminary conversion into

glycogen. Furthermore, in the large majority of cases we do not know the cause of such pancreatic lesions as may be found at autopsy. Sometimes there is evidently a chronic inflammatory process in the gland resulting from occlusion or infection of its duct, but usually this form of pancreatitis does not involve seriously the islands of Langerhans or cause diabetes. In the majority of the cases of diabetes in which pancreatic lesions are found the islands seem to be more affected than the other tissues,—indeed, they often appear to be specifically affected,—and the cause of this attack upon the islands is quite unknown.

The *metabolic processes* in diabetes seem also to be disturbed in quite the same way as after pancreatectomy, except in respect to the interference with digestion in the pancreatectomized animals because of the total absence of the pancreatic juice. (However, in some cases of human diabetes the pancreatic changes are so extensive as to interfere with the secretion of pancreatic juice.) Utilization of proteids and fats remains normal for some time, with excessive destruction of both to compensate for the lack of carbohydrate combustion, and a consequent increased elimination of nitrogen in the urine. Indeed, the amount of nitrogen eliminated may exceed that taken in with the food, because of the wasting in the tissues. This loss is commonly, although without conclusive proof, attributed to the action of poisonous substances upon the tissues, and called "*toxogenic proteid disintegration*." Associated with this tissue destruction is the presence in the urine of excessive quantities of purin bodies derived from the tissues (*endogenous purin bodies*). If all carbohydrates are eliminated from the diet, excretion of sugar may continue, indicating that sugar may be formed from proteids and fats. According to v. Noorden, synthesis of fat from carbohydrates is also impaired in most cases of diabetes, leading to wasting; but in some cases the synthesis of fat is not impaired, and in this event the fat tissues, being richly bathed with carbohydrates, build up excessive quantities of fat, leading to *obesity*. At first this formation of fat from the sugar may prevent glycosuria, but later the glycosuria appears, and we have the common form of "diabetes in the obese."¹

Loss of sugar and its consequences are not the only abnormalities from which diabetics suffer. The excessive quantity of

¹ According to Thoinot and Delamare (Presse Méd., 1904 (12), 491), pancreatic lesions are not present in cases of diabetes in the obese nor in nervous diabetes.

sugar in the blood seems to exercise a deleterious effect upon the tissues of the body, which is especially seen in the failure of repair in wounds. Slight injuries often lead to extensive tissue necrosis and gangrene. That this tendency to tissue disintegration and necrosis depends upon the hyperglycemia seems probable, because measures that are taken to reduce the amount of sugar in the blood exercise a favorable influence upon the tissue changes; however, we cannot be sure that unknown toxic materials are not also being reduced *pari passu* with the sugar. Furthermore, the amount of necrosis, gangrene, etc., of diabetes does not seem to bear a direct relation to the intensity of the hyperglycemia. However, there is no doubt that measures taken to keep down the amount of sugar in the blood and urine give diabetic patients the greatest freedom from complications and the longest duration of life, whether or not the sugar itself is the cause of their symptoms and sufferings.

v. Kossa has shown that *all varieties of sugar are toxic*, causing symptoms in many respects similar to those of diabetes when injected subcutaneously in doses of 1 per cent. (cane-sugar) of the body weight. Smaller doses, long continued, cause extreme emaciation with much loss of nitrogen and the development of nephritis. Albertoni observed increased heart action following injections of sugar, and Harley found that glucose injections caused the appearance of acetone bodies in the blood.¹ Scott² has claimed that glucose injections cause an increased elimination of nitrogen in forms other than urea, due to abnormal metabolism; this observation could not be corroborated by Underhill and Closson.³

Possibly *increased osmotic pressure* of the blood, because of the excess of sugar, plays an important rôle in the tissue changes; but this factor seems not to have been investigated, except for Pusey's experiments,⁴ which suggest the importance of osmotic pressure in the production of cataract, which is such a common result of diabetes.

Sweet⁵ has found that removal of the pancreas causes (in dogs) complete *loss of bactericidal power*, probably because of loss of bactericidal complement. The hemolytic power for foreign corpuscles is also greatly reduced through this loss of complement. It is quite possible that some similar impairment of bactericidal power explains the tendency to infection in diabetes.

¹ See Pflüger, Pflüger's Arch., 1903 (96), 376.

² Jour. of Physiol., 1902 (28), 107.

³ Jour. Biol. Chem., 1906 (2), 117.

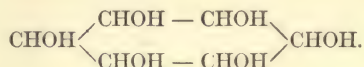
⁴ Arch. of Ophthalmology, 1904 (33), 128.

⁵ Jour. Med. Research, 1903 (10), 255.

"Bronzed Diabetes."—In this condition there appears a wide-spread deposition of an iron-containing pigment in the tissues and organs of the body, associated with the accumulation of an iron-free pigment in places where normally pigment is found in smaller amounts. This subject is discussed under "Pigmentation" (page 404). The diabetes is due to an interstitial pancreatitis, and must be considered as secondary to the disease, *hemochromatosis*, and not primary.¹ The cause of the disease is unknown.

CHRONIC POLYURIA

"Diabetes insipidus," which in some instances terminates in diabetes mellitus, but most generally seems to be quite distinct from true diabetes, presents little for consideration from the chemical side. Most striking, but by no means constant or characteristic, is the occurrence of *inosite* in the urine, sometimes in considerable quantities. Inosite, which occurs normally in the muscles, liver, spleen, kidneys, and other organs,² has been frequently found in the urine in both normal and pathological conditions. Although its empirical formula, $C_6H_{12}O_6$, is identical with that of the hexoses, yet it is a benzene derivative (Maquenne), having the following formula:



The normal constituents of the urine are generally increased in total amount, as if washed out with the excessive elimination of water. Consequently patients with this disease suffer from thirst and hunger, and drink and eat abnormally great quantities. Meyer³ states that the concentration of the urine tends to remain uniform, and that the amount of water is varied to regulate the concentration according to the amount of solids that are eliminated.

The etiology of the disease is unknown, but is probably various. Often it seems to be hereditary, but sometimes has been found associated with lesions in the pons, medulla, or cerebellum, which agrees with the observation of Bernard that experimental injuries of these parts may be followed by polyuria without glycosuria. In any case the increased flow of urine seems to be due to a dilatation of the vessels of the kidney, without increased arterial pressure;⁴ indeed, abnormally low blood pressure is often present. Presumably this vasodilatation depends upon nervous influences; a similar condition may be produced in animals by cutting the renal nerves.⁵

¹ See Opie, Jour. Exper. Med., 1899 (4), 279; Anschütz, Deut. Arch. klin. Med., 1899 (62), 411; Hess and Zurhelle, Zeit. klin. Med., 1905 (57), 344.

² See Meillère, "Inosurie. Recherche de l'inosite dans les tissus, les sécrétions et les excrétions." Paris, 1906.

³ Deut. Arch. klin. Med., 1905 (83), 1.

⁴ Review and literature by R. Schmidt, Wien. klin. Woch., 1905 (18), 1112.

⁵ Tallqvist (Zeit. klin. Med., 1903 (49), 181) on the basis of a study of the conditions of metabolism, suggests that the polyuria of diabetes insipidus may be due to a defective resorption of water in the renal tubules.

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